Low Dose Antithymocyte Globulin (ATG) to Delay or Prevent Progression to Stage 3 T1D

Protocol TN28

Version: 1.7

May 2, 2024

IND # 28654

NCT04291703

Sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

PREFACE

The Type 1 Diabetes TrialNet Protocol TN28, Low Dose ATG to Prevent Progression to Stage 3 T1D describes the background, design, and organization of the study.

The protocol will be maintained by the TrialNet Coordinating Center over the course of the study through new releases of the protocol, or issuance of updates either in the form of revisions of complete chapters or pages thereof, or in the form of supplemental protocol memoranda.

Glossary of Abbreviations

ADA	American Diabetes Association
AE	Adverse Event
AESI	Adverse Event of Special Interest
AGT	Abnormal Glucose Tolerance
ALT	Alanine Aminotransferase
ANCOVA	Analysis of covariance
APC	Antigen Presenting Cell
AST	Aspartate Aminotransferase
ATG	Anti-Thymocyte Globulin (Thymoglobulin®)
AUC	Area Under Curve
BB	BioBreeding
BMI-Z	Body Mass Index Score
CBC	Complete Blood Count
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practice
CGM	Continuous Glucose Monitor
CMV	Cytomegalovirus
COPD	Chronic Obstructive Pulmonary Disease
COVID-19	Coronavirus
CRF	Case report form
CRS	Cytokine Release Syndrome
DC	Dendritic Cells
DNA	Deoxyribonucleic Acid
DPTRS	DPT-1 (Diabetes Prevention Trial – Type 1) Risk Score
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr virus
FACS	Fluorescence activated cell sorting

FDA	US Food and Drug Administration
FWA	Federal-wide Assurance
GAD	Glutamate decarboxylase
GCP	Good Clinical Practice
GCSF	Granulocyte Colony Stimulating Factor
HbA1c	Hemoglobin A1c
HBsAg	Hepatitis B surface antigen
HLA	Human Leukocyte Antigen
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICA	Islet Cytoplasmic Antibodies
ICH	International Council for Harmonization
IEC	Independent Ethics Committee
IGRA	Interferon-γ release assays
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
ITN	Immune Tolerance Network
LIFT	Long Term Investigative Follow-Up
MAB+	Multiple Antibody Positive
MMTT	Mixed Meal Tolerance Test
NGT	Normal Glucose Tolerance
NIDDK	National Institute for Diabetes and Digestive and Kidney Disease
NIH	National Institute of Health
NCI-CTCAE	National Cancer Institute *Common Terminology Criteria for Adverse Events*
NOD	Nonobese diabetic
OGTT	Oral Glucose Tolerance Test
OHRP	Office for Human Research Protections

PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase chain reaction
PI	Principal Investigator
PK	Pharmacokinetic
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
PO	Per Oral (by mouth)
QA	Quality Assurance
RMSE	Residual Mean Square Error
RNA	Ribonucleic Acid
SAE	Serious adverse event
SOE	Schedule of events
SOP	Standard operating procedure
START	Study of Antithymocyte Globulin for Treatment of New-onset T1DM
T1D	Type 1 diabetes
Teff	Effector T cells
TN10	TrialNet Study 10 – Anti-CD3 Mab (Teplizumab) for Prevention of Diabetes in Relatives At-Risk for Type 1 Diabetes Mellitus
TN19	TrialNet Study 19 – Low Dose ATG vs ATG/GCSF vs Placebo in New Onset T1D
TNCC	TrialNet Coordinating Center
Tregs	Regulatory T cells
TSDR	Treg Specific Demethylation region
US	United States
ZnT8	Zinc Transporter 8

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1 INTRODUCTION

1.1 Study Overview

Title	Low-Dose ATG to Delay or Prevent Progression to Stage 3 T1D
IND Sponsor	National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Conducted By	Type 1 Diabetes Trial Network (TrialNet)
Protocol Chair	Michael J. Haller, MD
Accrual Objective	101
Study Design	A multi-center, placebo-controlled, double blind, 2:1 randomized control clinical trial testing low-dose ATG vs. placebo in participants with a 2-year 50% risk of progression to Stage 3 T1D.
Treatment Description	Participants will receive either low-dose ATG or placebo. Infusions will be given as follows: Day 1 of infusion will be ATG/Placebo at 0.5 mg/kg IV over a minimum of 6 hours. Day 2 of infusion will be ATG/Placebo at 2 mg/kg IV over a minimum of 6 hours. The maximum infusion time is 10 hours.
Study Duration	Enrollment is expected to occur over 4 years. Participants will be followed up for at least 24 months from randomization in this protocol. All participants that progress to Stage 3 T1D will be followed post-diagnosis for at least 12 months. All participants, regardless of T1D status will be followed for at least 12 months following the primary endpoint.
Objective	To determine the ability of low dose ATG to prevent or delay progression to Stage 3 T1D in participants aged 6-35 years with a 2-year 50% risk for progression.
Primary Outcome	The primary outcome is progression to the diagnosis of clinical T1D (Stage 3 T1D).
Secondary Outcomes	The study will also examine the effect of the proposed treatments on metabolic and immunologic markers of diabetes progression, continuous glucose monitoring metrics and adverse event frequency. Samples will be collected for mechanistic, PK and immunogenicity assays.
Major Inclusion Criteria	Participants will have a 2-year 50% risk for progression to Stage 3 T1D* defined by the presence of at least two biochemical autoantibodies, dysglycemia**, and at least one of the following additional high risk characteristics: • HbA1c ≥ 5.7% and <6.5% • Index60 ≥ 1.4 • DPTRS ≥ 7.4 *Dysglycemia and at least one high risk characteristic must be present at the same visit within 52 days of randomization. **Dysglycemia (ADA) is defined as 2-hr glucose ≥ 140 and <200 mg/dL or fasting glucose ≥ 110 and <126 or 30, 60, or 90 minute ≥ 200mg/dL.

2 BACKGROUND AND SIGNIFICANCE

2.1 Type 1 diabetes is an autoimmune disease that has its highest incidence in childhood

Type 1 diabetes (T1D) has been recognized to be an autoimmune disease on the basis of studies in persons with the disease, individuals at-risk who progress to overt disease, and the effects of immune-directed therapies on the disease progression. Preclinical models, which share many features with human T1D, have been used to elucidate the disease mechanisms and to develop therapies that might be used to treat or prevent the autoimmune process. Most data suggest that autoreactive T cells are involved as effector cells and that B lymphocytes also play a role.

T1D is one of the most common chronic diseases of childhood and the incidence has been increasing. Although T1D may occur at any age, the peak incidence of T1D is in children – between the ages of 6-12 with a second peak later in adolescence. In the US, one longitudinal study between 2001 and 2015 found that the incidence rate of T1D in children was 45.5 in children aged 10-14 years and 18.6 per 100,000 in adults ages 20-64[3]. The clinical presentation of T1D in adults is milder than in children: the hallmark of new onset T1D, diabetic ketoacidosis, is less common. This most likely reflects the greater residual beta cell function at the time of diagnosis in adults and slower rate of progression of the disease in older persons. Indeed, even data from relatives at risk for T1D has shown a slower rate of disease progression in adults.

2.2 Introduction to T1D Staging, T1D TrialNet, and T1D Risk Determination

T1D Staging: *T1D is a chronic autoimmune disease that develops over years:* Beginning with clinical observational studies in the 1970s, an understanding of T1D as a chronic autoimmune disease has developed[4]. Specifically, it is recognized in the American Diabetes Association's (ADA) 2024 Standards of Medical Care that there are three distinct stages of type 1 diabetes. These stages provide an important framework for T1D clinical care, research, and regulatory decision-making[5-7] (Table 1).

	Stage 1	Stage 2	Stage 3
Characteristics	• Autoimmunity • Normoglycemia • Presymptomatic	• Autoimmunity • Dysglycemia • Presymptomatic	• Autoimmunity • Overt hyperglycemia • Symptomatic
Diagnostic criteria	• Multiple islet autoantibodies • No IGT or IFG	• Islet autoantibodies (usually multiple) • Dysglycemia: IFG and/or IGT • FPG 100–125 mg/dL (5.6–6.9 mmol/L) • 2-h PG 140–199 mg/dL (7.8–11.0 mmol/L) • A1C 5.7–6.4% (39–47 mmol/mol) or ≥10% increase in A1C	• Autoantibodies may become absent • Diabetes by standard criteria

Adapted from Skyler et al. (40). FPG, fasting plasma glucose; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; 2-h PG, 2-h plasma glucose. Alternative additional stage 2 diagnostic criteria of 30-, 60-, or 90-min plasma glucose on oral glucose tolerance test ≥200 mg/dL (≥11.1 mmol/L) and confirmatory testing in those aged ≥18 years have been used in clinical trials (79).

Table 1: Stages of Type 1 diabetes (from Diabetes Care vol 47 Supplement 1, Jan 2024)

Markers of beta cell autoimmunity, such as autoantibodies or islet antigen reactive T cells, are observed in persons developing diabetes long before they present with clinical disease[8,9]. In addition, abnormalities in insulin secretion patterns and even glucose intolerance, are found before clinical presentation (Stage 3)[6,10,11]. Notably, the 2024 ADA Standards of Medical Care recognize that in research, stage 2 diagnostic criteria include 30, 60 and 90 minute values of over 200 mg/dl during a standard 2-hour oral glucose tolerance test[7]. The inclusion of the 30, 60, 90-minute timepoints is consistent with multiple Trialnet publications demonstrating that glucose values at or above the diagnostic values at any of these intermediary timepoints denotes high risk of progression to clinical disease. In the Insel et al paper and demonstrated in Figure 1, where the reference uses the labels impaired fasting glucose (IFG), impaired glucose tolerance (IGT) at 120-minutes and indeterminate values at 30,60 or 90 minutes in showing similar proportions without T1D in comparison to those with normal glucose tolerance. Abnormal glucose at any point in the 2-hour glucose tolerance test defines high risk for clinical disease. Additionaly, findings from Larsson et al demonstrated that maximal plasma values equal to or greater than 11.1 mmol/L at the intermediariy OGTT timepoints predicted development of diabetes in 10 out of 12 children in the study[12]. The inclusion of intermediary timepoints for trial eligiblity criteria from oral glucose tolerance testing are essential in identifying additional participants at risk for progression to Stage 3 T1D.

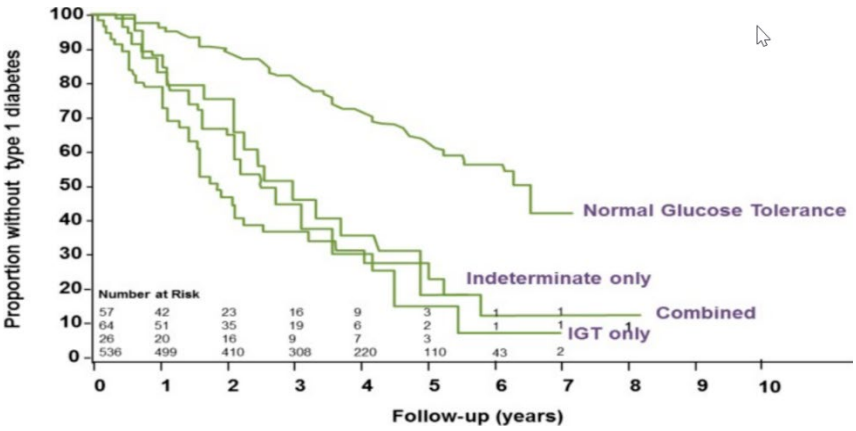

Figure 1: Probability of progression from dysclycemia stage 2 in DPT-1. IGT, impaired glucose tolerance.

The immune mechanisms leading to the decline in beta cell function *before* clinical diagnosis are thought to be the same as those that account for the ongoing loss of function *after* the formal disease diagnosis [13]. The Stages of T1D therefore do not represent a difference in the pathologic process, rather they are defined on the basis of glucose levels, associated symptoms, and/or elevations in hemoglobin A1c levels. There is, by definition, much greater beta cell function in people with Stage 2 versus Stage 3 diabetes[14,15]. As such, if immune therapies are successful at arresting or slowing beta cell loss, there is an even greater potential for clinical benefit in Stage 2 T1D, since effective therapies would increase the time without a requirement for exogenous insulin administration, continuous monitoring of glucose levels, and constraints on activity and diet. Particularly for children, in whom the incidence of the disease is greatest, and their families, there is clear benefit in delaying the diagnosis of Stage 3 T1D.

TrialNet: TrialNet was formed in 2001 to create the infrastructure needed to test ways that T1D could be delayed or prevented in individuals destined to be diagnosed with clinical stage 3 T1D. Prevention, rather than treatment after the diagnosis of disease, was then and largely remains a novel strategy for averting autoimmunity. Over time, this notion has found increasing support due to our evolving understanding that clinical T1D is a chronic, rather than acute autoimmune disease. T1D begins with the appearance of 2 or more autoantibodies and progresses through overt beta cell destruction until metabolic demands cannot be met and glycemic decompensation occurs.

This understanding of T1D pathogenesis has built the framework for TrialNet to identify individuals at high risk for clinical disease and to perform clinical studies to identify therapies capable of delaying or preventing clinical onset (**Fig 2**). Often, TrialNet has used a stepwise approach, based initially on preclinical studies followed by clinical outcomes observed in therapeutic trials involving people within 100 days of diagnosis of Stage 3 diabetes. If evidence of efficacy (i.e., a positive

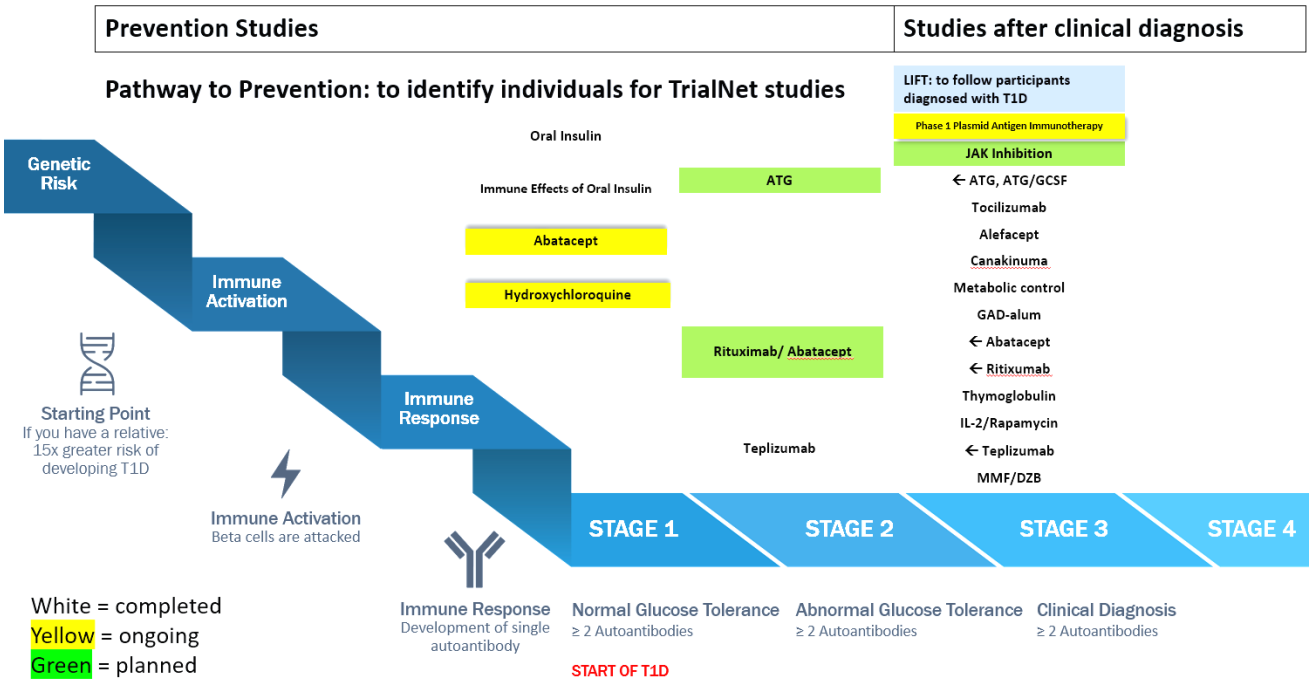

Figure 2: TrialNet's workflow for identifying promising candidates for prevention of T1D. The ribbon diagram illustrates the designated stages in the development of T1D. Once a relative is found to have 2 or more autoantibodies, they are designated as having Stage 1 diabetes and on a pathway to diagnosis. They are followed in the "Pathway to Prevention" study. TrialNet frequently conducts studies in new onset Stage 3 diabetes (within the first 100 days after diagnosis). Drugs that show efficacy in Stage 3 T1D (based on evaluation of beta cell function with C-peptide and clinical parameters), shown in **bold** are then moved into prevention efforts in Stage 1 and Stage 2 (arrows). The same pathway is proposed for ATG. (Some studies have been done in collaboration with other consortia.)

signal in terms of either metabolic progression or a favorable immune system marker) is observed in people who had recently been diagnosed with stage 3 T1D, TrialNet would then move to testing the same drugs in Stage 2 T1D.

This stepwise approach was used for development of anti-CD3 mAb, teplizumab. Initially studies were performed in preclinical (murine) models of autoimmune diabetes – with multiple low doses of streptozotocin and in NOD mice[16,17]. Based on these studies and the production of teplizumab, a humanized anti-CD3 mAb, clinical trials were initiated in adults and children, as young as age 8, with Stage 3 disease[18-20]. Based on those trials, TrialNet conducted the TN10 study in persons with abnormal glucose tolerance to include the 30, 60, and 90 minute OGTT timepoints and multiple autoantibodies to test whether teplizumab would delay or prevent progression to Stage 3. That study randomized people to placebo or teplizumab and showed that a single course of teplizumab delayed Stage 3 diabetes for a median of 2.7 years[21]. Based largely on these results, teplizumab was approved by the FDA in late 2022 to delay the onset of Stage 3

type 1 diabetes in adults and pediatric patients 8 years and older with Stage 2 T1D. As such, for all current and future studies enrolling participants who might be eligible for teplizumab as a clinical FDA approved therapy, additional effort must be taken to ensure participants have a complete understanding of their therapeutic and research opportunities during the consent process.

While not addressed in detail here, a similar approach was used for the design and eventual implementation of studies using abatacept in adults and children in which benefit was initially shown in those with Stage 3 disease. With those supportive data in hand, TrialNet was given approval to enroll and complete a study of both children and adults seeking to delay progression from Stage 1 to Stage 2 T1D. In terms of issues related to prospect of direct benefit, it is notable the TrialNet abatacept Stage 1 study was FDA approved without a requirement to first demonstrate a delay in progression from stage 1 to stage 2 T1D in adults[22,23].

Biochemical and metabolic markers identify study participants who have a high risk of progression to clinical (Stage 3) T1D: Since March 2004, the TrialNet Pathway to Prevention (TNPTP) study has collected assessments over time from individuals at risk of developing T1D. Assessments have included T1D-related autoantibodies, oral glucose tolerance tests, HbA1c, clinical measures, and assessments of T1D diagnosis. Current TNPTP data show 30% of individuals at Stage 2 progress to Stage 3 within two years (**Fig 3**). Based on the rationale that (1) the stages of T1D represent a continuum of the same disease and (2) therapies capable of altering the course of Stage 3 diabetes may be effective if applied at Stage 1 or 2, TrialNet set forward a series of studies to interdict the process and delay progression from Stage 1 or 2 to Stage 3 T1D[4,18,24-29].

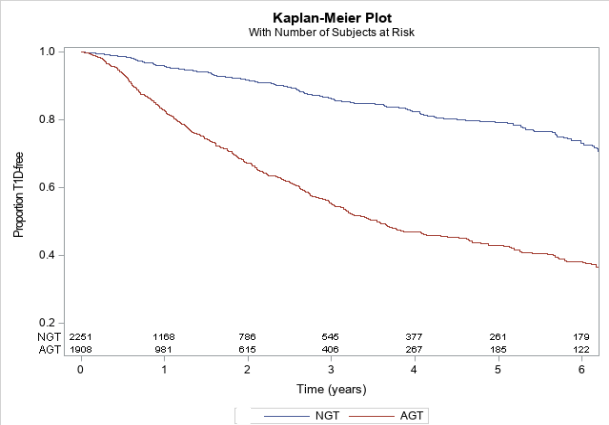

Figure 3: Proportion of participants who have 2+ autoantibodies and are T1D-free and have normal glucose tolerance (NGT) vs abnormal glucose tolerance (AGT) over time.

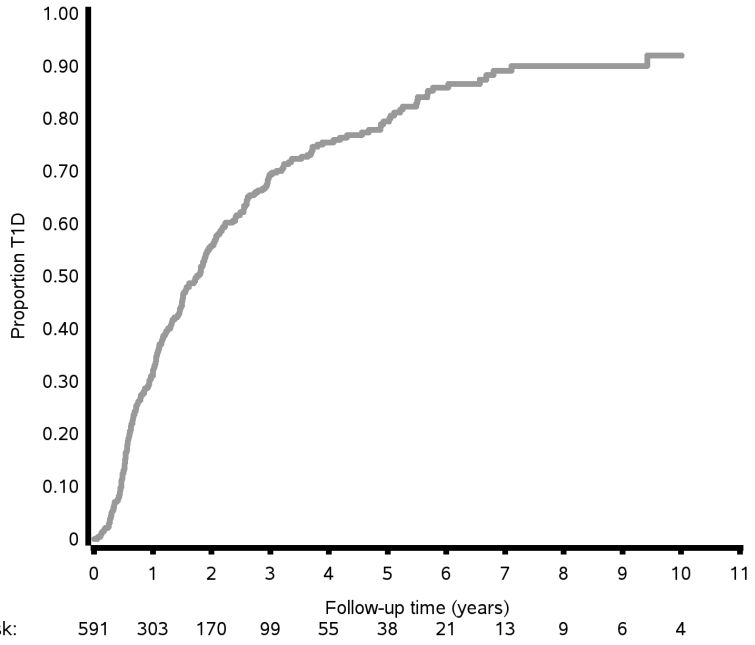

Figure 4: Kaplan-Meier Curve showing Cumulative T1D Incidence over time in TN01 participants ages 6-35, MAB+ with an abnormal OGTT and at least one high risk marker.

Data about the risk for progression from Stage 2 to Stage 3 diabetes have largely been developed from pediatric longitudinal studies. In most individuals who ultimately progress to Stage 3 diabetes, autoantibodies first appear in early childhood. Based on serial assessments of beta cell function (i.e. stimulated C-peptide levels (with oral glucose tolerance tests), children progress through the stages of T1D more rapidly than in adults. Our experience in following participants with Stage 2 diabetes in the TrialNet Pathway to Prevention study (defined based on abnormal glucose tolerance at times 0, 30, 60 90 or 120 minutes during an OGTT for participants between 6 and 35 years old with HbA1c 5.7 - <6.5 OR Index60 ≥ 1.4 OR DPTRS ≥ 7.4) illustrates this point (**Fig 4**). Thus, once identified, the 5-year risk of Stage 3 diabetes is greater for children with abnormal glucose tolerance and multiple autoantibodies compared to adults.

T1D Risk Determination: While TrialNet and the larger T1D community successfully used staging to help patients, providers, and regulatory agencies understand the natural history of pre-clinical T1D, TrialNet and other prospective longitudinal studies[12,30,31], as introduded above, now able to more precisely define populations at risk for developing Stage 3. Specifically, using TrialNet OGTT data we identified markers defining cohorts with a 2-year 50% risk of progressing to Stage 3 T1D. Based on these analyses, 2-yr High-Risk (50% risk for progression to Stage 3 T1D) criteria were defined as ever having two or more T1D-associated islet autoantibodies, ADA Stage 2 criteria (dysglycemia defined as impaired fasting plasma glucose of ≥100 mg/dL (≥5.6 mmol/L), impaired glucose tolerance (a 2-h plasma glucose of ≥140 mg/dL (≥7.8 mmol/L), or high glucose levels at intermediate time OGTT points (30, 60, 90 min levels of ≥200 mg/dL [≥11.1 mmol/L]) and meeting at least 1 of the following characteristics on one visit: HbA1c ≥ 5.7% and < 6.5% or Index60 ≥ 1.4 or DPTRS ≥ 7.4[12,30,31].

To reiterate, while staging inherently predicts progression to disease (e.g. Stage 2 broadly provides a 5 year 50% progression risk), the use of additional risk markers routinely obtained during TrialNet OGTTs identifies sub-populations with even greater short term (2-year 50%) risk of progression to Stage 3 T1D. These highest risk participants are optimal for studies in prevention / delay as studies

in this space rely on event rates of individuals progressing to Stage 3 T1D rather than a time related endpoint (e.g. 1 year post treatment)[32]. As such, this protocol acknowledges and uses staging but goes further to rely on additional risk markers to define a study population of participants with a 2 year 50% risk of progression.

2.3 Rationale for ATG Therapy in T1D

Immunotherapies, including ATG, can modify the disease course and even delay or prevent progression from stage 2 to stage 3 T1D.[33-35]. The rationale for this proposed trial is based on preclinical and clinical studies as outlined below.

Studies of ATG in murine T1D models: In NOD mice, a preclinical model of spontaneous autoimmune diabetes, treatment with ATG reversed the overt hyperglycemia used to diagnose diabetes (i.e. a glucose > 250 mg/dl x 2) (**Fig 5 D, E**). Moreover, treatment of mice at 12 weeks of age prevented the diabetes onset (**Fig 5 C**). The ATG treatment was effective for prevention at 12 but not at earlier times (4 and 8 weeks) (**Fig 5 A, B**).

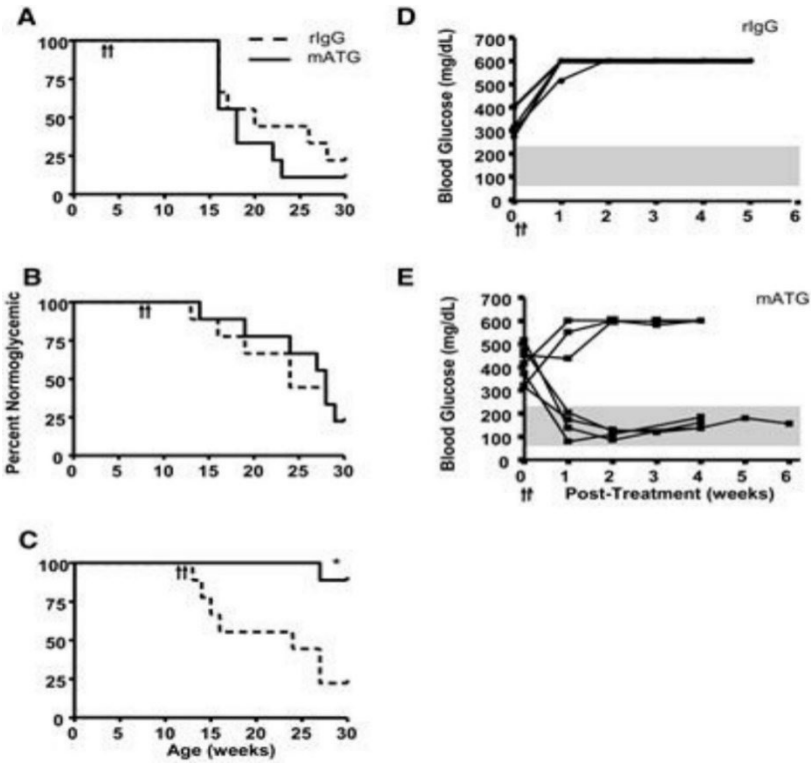

Figure 5: Development of type 1 diabetes in NOD mice is delayed by mATG in a time-dependent manner, ATG was administered to female NOD mice age 4 (A), 8 (B), 12 (C) weeks of age (mATG, (solid line) or rIgG, (dashed line) (1.0 mg/animal [two 500 µg doses 72 h apart]) and followed for development of diabetes until 30 weeks of age (A-C)(n = 9 per group)(*P < 0.004, by Kaplan-Meier analysis). In addition, treatment with ATG (E) but not rIgG (D) reversed diabetes in NOD mice with new onset hyperglycemia. Blood glucose levels (non-fasting) in nondiabetic NOD mice from the same colony are shaded in gray[1]

These findings were replicated in other studies in NOD mice[36,37]. These preclinical studies highlight the importance of the timing of intervention with ATG: prevention of overt hyperglycemia was best at a relatively late time (12 weeks) during the progression of the disease. Insulitis begins at about 6 weeks in NOD mice, dysglycemia appears at 10-13 weeks of age, and hyperglycemia after that time (**Fig 6**)[38]. Thus, these studies support our proposed intervention with ATG during Stage 2 diabetes in humans.

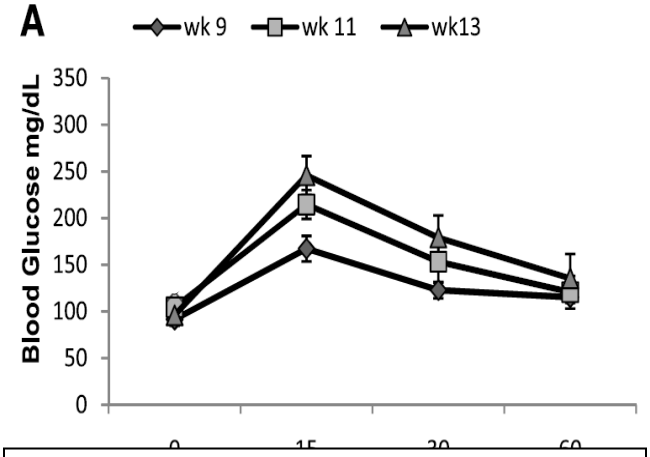

Figure 6: Glucose tolerance in prediabetic NOD mice. Female NOD mice at ages 9, 11, and 13 weeks underwent a standard ipGTT and glucose levels in the peripheral blood were measured (n=5/group). The mean (SEM) glucose levels at 15, 30, and 60 min after the glucose challenge are shown.

Clinical experience with ATG in persons with Stage 3 diabetes: Initial studies with equine ATG and prednisone in study participants with new onset T1D suggested efficacy in prolonging the "honeymoon" phase period of improvement in metabolic control after the diagnosis of T1D[39,40]. Similarly, in a small randomized, placebo-controlled, single-masked trial with ATG (ATG-Fresenius, Germany), seventeen participants with T1D aged 18-35 years received a total dose of 18 mg/kg of ATG, divided across in four infusions (n=11) or placebo (n=6). The ATG group, compared to the placebo group, had increased glucagon-stimulated C-peptide levels, a lower insulin requirement, and lower glycosylated hemoglobin levels at the 12-month study visit. Notably, two ATG-Fresenius treated participants did not require exogenous insulin for 4 and 13 months, respectively, with fasting blood glucose below 126 mg/dl after treatment[41].

A subsequent Immune Tolerance Network study of the Sanofi ATG product (Thymoglobulin) at a lower dose of 6.5 mg/kg was performed in 58 participants with recent onset stage 3 T1D between the age of 12 and 35 but failed to show significant improvement in C-peptide compared to controls[42]. That said, a post-hoc analysis suggested C-peptide responses were preserved among the young adult cohort and gave support to the hypothesis that ATG could still be beneficial if given at the right dose and to the right subset of people with diabetes.

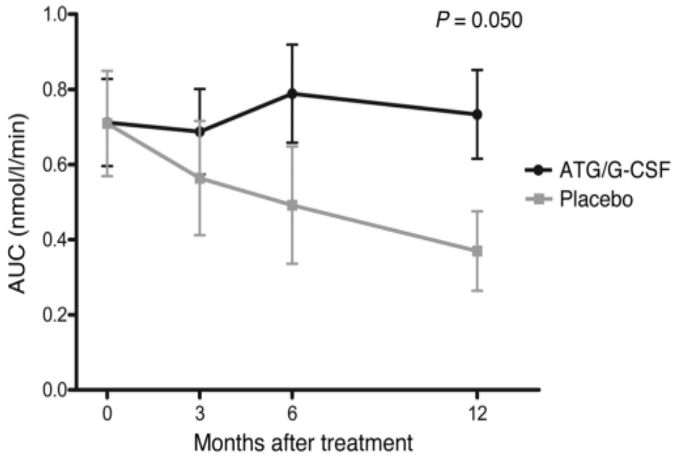

Figure 7: AUC-peptide of ATG/GCSF vs Placebo participants at 1-year post treatment. 2-Sample, 2-sided t test showing mean +/- SD

In an effort to determine if even lower dose ATG might differentially target effector and regulatory T cells and in order to determine if a second agent, GCSF, might support synergy by promoting regulatory T cell function, the University of Florida, University of Colorado, and University of California San Francisco teams subsequently lead a study of lower dose of ATG (2.5mg/kg) in combination with GCSF in persons with established T1D. Notably, the dose of 2.5mg/kg of ATG is 5-6 times lower than that commonly used in renal

transplantation and rejection and was chosen to both reduce risk of immunosuppression and explore the differential effect of low-dose ATG on regulatory T cell function. This randomized single-blind trial of 25 persons between 12 and 45 years of age (10 participants aged 12-18) with recent-onset (within 12 months) stage 3 diabetes analyzed changes in C-peptide responses to a mixed meal tolerance test and the HbA1c levels[43]. The MMTTs were used for assessment of beta cell function, measured by C-peptide in people after the diagnosis of Stage 3 T1D (Note: oral glucose tolerance tests (OGTT) are used to diagnose diabetes and to assess beta cell function prior to diagnosis.) In this pilot study, C-peptide responses and HbA1c levels were improved at 1 year after randomization and treatment.

Importantly, the adverse events experienced with low-dose ATG were expected, transient and manageable with glucocorticoids and analgesics. There were no differences in AEs between children and adults.

TrialNet completed a randomized placebo-controlled trial of low-dose ATG in persons with new-onset Stage 3 diabetes (TN19), and compared ATG+GCSF (n=29) or ATG (n=29) to placebo (n=31)[5] . Participants in TN19 received 2.5mg/kg ATG, or 2.5mg/kg ATG plus GCSF, or placebo and were followed for 2 years. As discussed in detail below, the study found no additional benefit associated with adding GCSF to ATG[44]. As such, only ATG and placebo data from the TN19 trial are discussed below and the study outlined in the protocol below, TN28, will utilize only ATG monotherapy versus placebo.

At 1 year after study entry, the group treated with ATG showed a significant improvement in the C-peptide response (mean area under the curve (AUC)) to MMTT (0.646, CI 0.55, 0.75) nmol/L) versus placebo (0.406, CI 0.32, 0.49) nmol/L) (p=0.0003) (**Fig 8**). In addition, the HbA1c was significantly reduced at 1 year in participants treated with ATG compared to placebo (p=0.002) (**Fig 9**). All results were adjusted for age and baseline C-peptide responses). At month 24 the HbA1c levels were significantly lower and the AUC of C-peptide responses were significantly greater in the ATG vs placebo groups (p=0.01 and p=0.00005, respectively)[5].

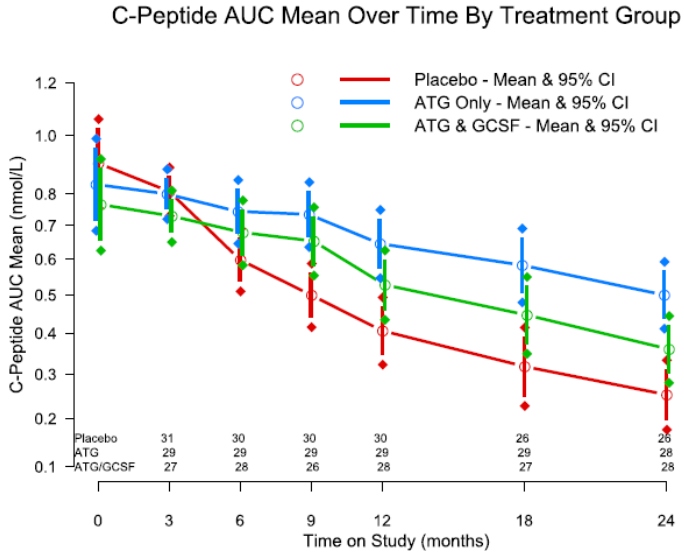

Figure 8: Effects of low-dose ATG treatment of patients with Stage 3 diabetes on C-peptide responses obtained by MMTT (TN19). (p=0.0003 and 0.00005 at 1 and 2 yrs after the C-peptide responses were corrected for the baseline and age)

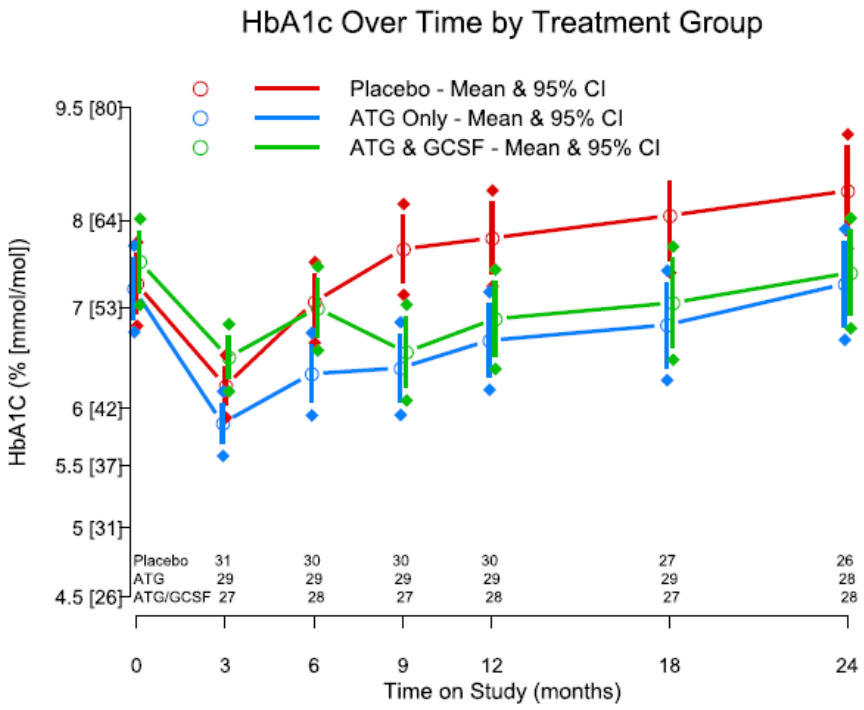

Figure 9: Effects of low-dose ATG treatment of patients with Stage 3 diabetes on HbA1c levels in the TN19 trial.

Most recently, in a case study using off-label ATG treatment in 6 children aged 5 -14 years with Stage 2 T1D, 3 of the participants have not progressed to Stage 3 T1D after 1.5, 3 and 4 years of follow-up[2]. The remaining 3 participants developed Stage 3 T1D but demonstrated low insulin requirements, near normal HbA1c, preserved C-peptide values and high time in range metrics for the remainder of the 18 months of post ATG follow-up[2]. Participants were dosed 2.5 mg/kg over two infusions (day 1, 0.5 mg/kg; day 2, 2.0 mg/kg). All participants experienced grade 3 serum sickness (SS) and in half of the participants, grade 1 cytokine release syndrome (CRS) was observed. Through use of the appropriate medications, participant's symptoms were alleviated and/or resolved.

In summary, preclinical studies, in mice with diabetes and prediabetes, and a series of clinical studies in persons with Stage 3 diabetes, have established that ATG preserves C-peptide in humans with Stage 3 diabetes and in mice before the onset of hyperglycemia. Notably, the benefits of low-dose ATG in persons with T1D (A1c reduction, C-peptide preservation) persisted for more than 2 years after a single 2-day course of treatment. The case study provides critical experience in the use of ATG to delay progression of stage 2 T1D in children as young as 5 years and demonstrates side effect tolerability with the appropriate medications[2]. Although not statistically powered, half of the case study participants did not progress to Stage 3 T1D as far out as 4 years

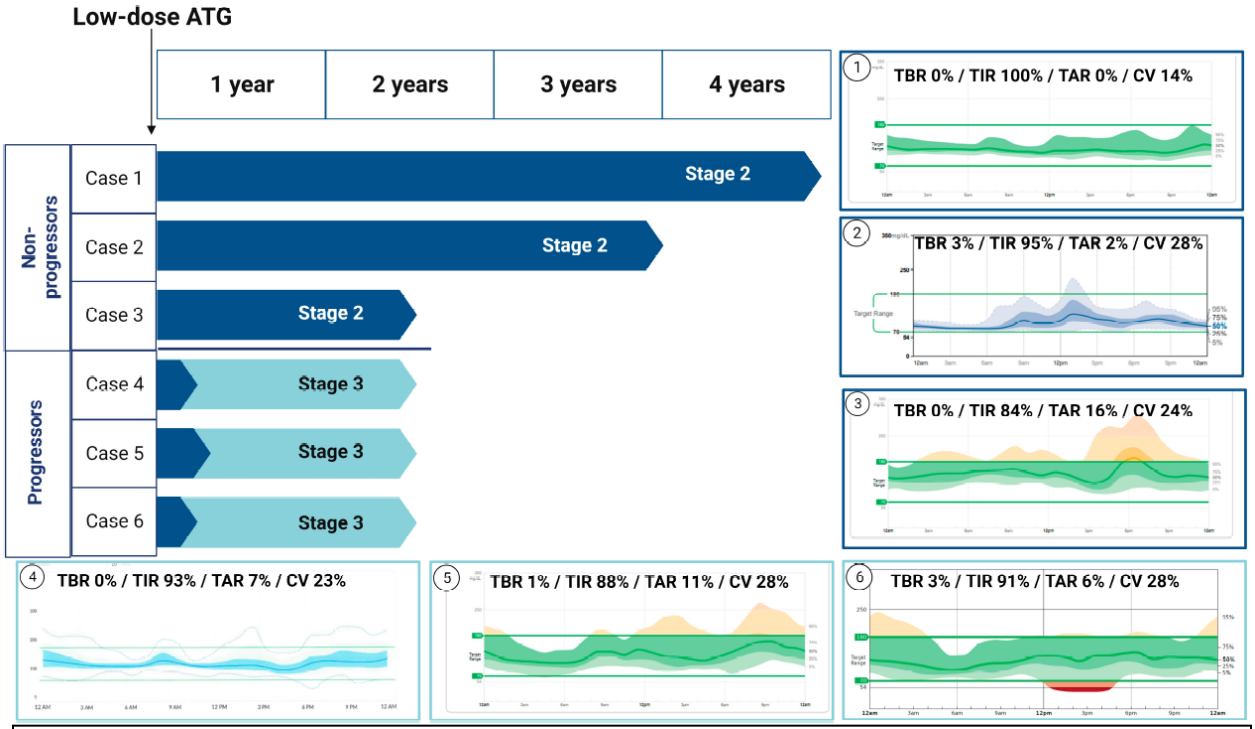

Figure 10: Progression versus nonprogression and CGM metrics in stage 2 ATG recipients. Blue bars demonstrate duration of delay as well as total duration of follow-up (upper left). CGM tracings for each subject are shown over a 2-week period at their final documented visit to date, indicating high TIR, low time below range (TBR), low time above range (TAR), and low coefficient of variation (CV) in both nonprogressors (cases 1–3, right) and progressors (cases 4–6, lower)[2].

from initial treatment (Fig 10). This, in combination of the larger data set related to the use of low-dose ATG in T1D provides demonstration of prospect of benefit to pediatric participants with Stage 2 T1D. As Stage 2 and Stage 3 diabetes represent the same pathologic process on the continuum of disease progression, the data strongly support testing the ability of ATG to prevent or delay the onset of clinical T1D in adults and children with Stage 2 T1D who are at high risk of rapid progression to stage 3 disease [14,44-46].

2.3.1 Potential Mechanism of Action

ATG appears to induce both generalized immunosuppression and immunoregulation[47]. The drug consists of polyclonal antibodies against thymocytes and also targets other immune cells. It prevents B-cell proliferation and differentiation and mediates T-cell suppressive effects via inhibition of proliferative responses to mitogens[48,49].

Preclinical data, although limited, support the mechanisms observed in study participants, and demonstrate that low-dose ATG has multiple beneficial mechanistic actions (e.g., increased Treg:Teff ratios, reduced islet inflammation, improved beta cell area, etc.). Notably, early changes in the Treg/Teff ratio (at 3 months post therapy) were predictive of response to therapy (AUC C-peptide) at 2 years in the TN19 clinical trial

In humans, a “standard dose” of ATG (10-15 mg/kg) reduces the total lymphocyte count by more than 85%. T-cell depletion may result from complement-dependent opsonization and cellular lysis, Fc-dependent opsonization, or Fas-mediated apoptosis. Fas-mediated apoptosis is likely the more predominant mechanism at lower ATG concentrations, where ATG exhibits preferential effects on pre-activated, as opposed to nonactivated, T-cells. It has been hypothesized that ATG administration may induce a population of CD8+, CD57+CD28- T cells that have regulatory effects on CD4+ Th1 cells that may enable autoimmune destruction of beta cells[44]. Total lymphocyte and CD8+ T cell counts typically return to normal within 2 months of ATG administration, whereas CD4+ T cell counts can remain reduced for 12 months. These effects may explain the lasting immune-modulatory activity of ATG. Following ATG infusion, the CD4:CD8 T cell ratio remains significantly and persistently lower, relative to baseline values, and the CD4:CD8 ratios often correlate with graft survival and rejection rates in transplantation studies[50-53].

Other studies have suggested alternative mechanisms underlying the beneficial ATG effects. Like anti-CD3, ATG may induce partial T-cell activation, leading to an anergic state[54-57]. In cynomolgus monkeys, ATG appears to coat T cells, leading to downregulated surface expression of CD2, CD3, CD4, and CD8 molecules, along with impaired immune responses in mixed lymphocyte reactions[36]. In addition, antibodies to adhesion molecules may interfere with cellular adhesion and endothelial interactions, as well as T-cell migration to sites of inflammation. ATG may also prevent T cell co-stimulation by binding directly to APC, and may induce complement-mediated lysis of these cells; particularly in more mature APC[58]. The survival of immature dendritic cells (DC) thus may be more tolerogenic. Antigens recognized by ATG include CD86, CD32, CD4, CD11b, CD29, and CD51/61; some of which are shared by lymphocytes and DC. In addition, ATG contains antibodies that cross-react with B-cell surface antigens, promoting activated B cell apoptosis[59-61].

2.3.2 Safety and efficacy experience of ATG in Children with Stage 3 T1D: Prospect of direct benefit for children with Stage 2 T1D

ATG has been standard of care for induction therapy for pediatric renal transplant recipients for nearly 20 years and has been administered to hundreds of people at doses that are 5-7 times higher (10-15mf/kg) than is proposed for treatment of Stage 2 diabetes in this protocol (2.5mg/kd)[62]. There is also extensive experience with its use in children for treatment of transplant rejection[63]. The North American Pediatric Renal Trials Collaborative Studies registry (n=455) demonstrated that doses of <7.5mg/kg of ATG prevent renal transplant loss safely and effectively[64]. Similarly, a study of 235 pediatric renal transplant recipients following ATG at doses < 4.5mg/kg revealed the even lower dose provided safe and effective immunosuppression versus higher doses[65]. The adverse events were managed with standard therapies used in other settings. At the proposed and higher doses, the adverse events were reversible.

The TrialNet TN19 trial enrolled participants with new onset Stage 3 T1D between the ages of 12-45 years and was not powered to compare age groups as a pre-specified analysis. However, in a post-hoc analysis comparing participants less then or greater than age 18, the C-peptide and HbA1c responses were at least as robust, if not more so, in children versus adults (**Fig 11)**. This provides additional strong support for prospect of benefit when considering the use of low-dose ATG to prevent progression from Stage 2 to Stage 3 T1D in children.

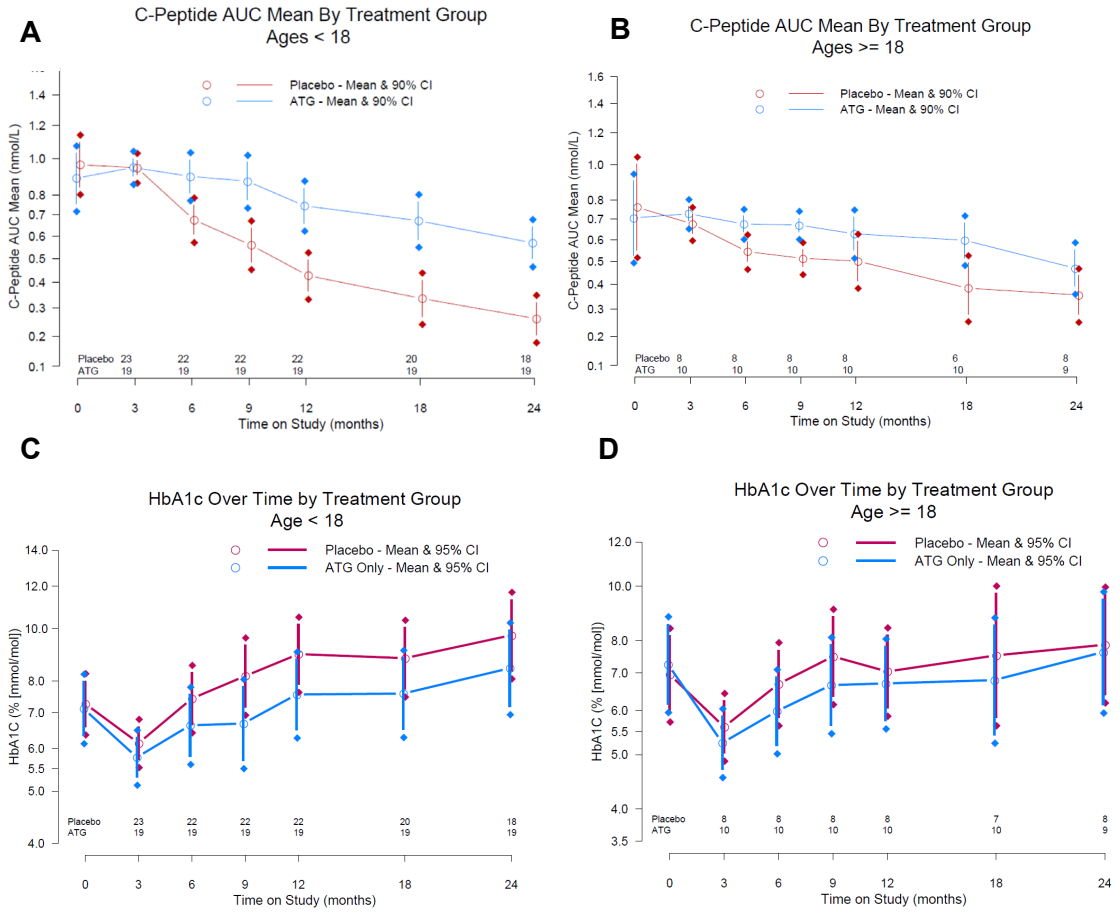

Figure 11: C-peptide responses (A, B) and HbA1c levels (C, D) in children (A, C) and adults (B,D) in the TrialNet TN19 study.

Adverse events previously reported in studies of low dose (2.5 mg/kg) ATG in new-onset T1D (ages 12–45 years) included transient cytokine release syndrome (CRS), lymphopenia and serum sickness[66]. Notably, these side effects were similar in frequency and severity when comparing children to adults in the TN19 study. There were no reported cases of diabetic ketoacidosis nor severe hypoglycemia in the TN19 trial participants[44,66].

In the adult population (18-45 years) of participants treated with ATG monotherapy (n=10) in the TN19 study, no serious adverse events were reported. 51 non-serious adverse events were reported in adult receiving ATG monotherapy. 75.5% of those events were assessed as related to ATG treatment. The most commonly reported non-serious adverse events in adults were decreased lymphocyte count, serum sickness, fever and cytokine release syndrome (Table 2).

TABLE 2: Non-Serious Adverse Events Reported in TN19 Study for Participants Ages 18-45					
		Attribution #			
Systems Affected (CTC 4.0)		Non-serious - related		Non-serious - unrelated	
Category	AE term	Number of Participants	Number of Adverse Events	Number of Participants	Number of Adverse Events
Endocrine disorders	Hypothyroidism	0	0	1	1
General disorders and administration site conditions	Fever	2	4	0	0
	General disorders and administration site conditions - Other, specify	1	3	0	0
	Infusion site extravasation	1	1	0	0
Immune system disorders	Cytokine release syndrome	3	3	0	0
	Serum sickness	7	7	0	0
Infections and infestations	Sinusitis	1	1	0	0
	Upper respiratory infection	1	2	1	1
Investigations	CD4 lymphocytes decreased	5	5	0	0
	Lymphocyte count decreased	8	8	0	0
Metabolism and nutrition disorders	Metabolism and nutrition disorders - Other, specify	0	0	1	2
Musculoskeletal and connective tissue disorders	Arthralgia	1	1	0	0
	Bone pain	1	1	0	0
	Myalgia	2	2	0	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other, specify	0	0	1	1
Nervous system disorders	Tremor	0	0	1	1
Psychiatric disorders	Depression	0	0	1	1
Respiratory, thoracic and mediastinal disorders	Cough	0	0	2	2
	Nasal congestion	0	0	1	1
Skin and subcutaneous tissue disorders	Skin and subcutaneous tissue disorders - Other, specify	0	0	2	2
Surgical and medical procedures	Surgical and medical procedures - Other, specify	0	0	1	1

In the pediatric population (12-17) of participants treated with ATG only, a total of 4 serious adverse events were reported with only 2 considered related to study agent (ATG); 1 report of vasovagal reaction and 1 report of serum sickness (Table 3). All adverse events reported for the ATG only treated participants from the TN19 study (serious and non-serious) are included in the TrialNet IB addendum.

TABLE 3: Serious Adverse Events Reported in TN19 Study for Participants Ages 12-17					
		Attribution #			
Systems Affected (CTC 4.0)		Serious - related		Serious - unrelated	
Category	AE term	Number of Participants	Number of Adverse Events	Number of Participants	Number of Adverse Events
Immune system disorders	**Serum sickness**	1	1	0	0
Injury, poisoning and procedural complications	**Injury, poisoning and procedural complications - Other, specify**	0	0	1	1
Nervous system disorders	**Vasovagal reaction**	1	1	0	0
Surgical and medical procedures	**Surgical and medical procedures - Other, specify**	0	0	1	1

In the pediatric population, 115 non-serious adverse events were reported. Of those 115 non-serious adverse events, 89 were assessed as related to ATG treatment (77.4%). The most commonly reported non-serious adverse events in the pediatric population were serum sickness, cytokine release syndrome, decreased lymphocyte counts, headache and decreased white blood cell count (Table 4).

TABLE 4: Non-Serious Adverse Events Reported in TN19 Study for Participants Ages 12-17					
		Attribution #			
Systems Affected (CTC 4.0)		Non-serious - related		Non-serious - unrelated	
Category	AE term	Number of Participants	Number of Adverse Events	Number of Participants	Number of Adverse Events
Blood and lymphatic system disorders	Blood and lymphatic system disorders - Other, specify	1	1	0	0
Gastrointestinal disorders	Abdominal pain	2	3	0	0
	Dyspepsia	1	1	0	0
	Nausea	1	1	0	0
	Toothache	0	0	1	2
	Vomiting	0	0	2	2
General disorders and administration site conditions	Chills	1	1	0	0
	Fatigue	1	1	0	0
	Fever	2	2	0	0
	Flu like symptoms	2	3	0	0
	General disorders and administration site conditions - Other, specify	1	1	1	1
	Pain	2	2	0	0
Immune system disorders	Cytokine release syndrome	11	14	0	0
	Serum sickness	13	13	0	0
Infections and infestations	Otitis media	0	0	1	1
	Pharyngitis	1	1	0	0

TABLE 4: Non-Serious Adverse Events Reported in TN19 Study for Participants Ages 12-17					
		Attribution #			
Systems Affected (CTC 4.0)		Non-serious - related		Non-serious - unrelated	
Category	AE term	Number of Participants	Number of Adverse Events	Number of Participants	Number of Adverse Events
	Rash pustular	0	0	1	1
	Skin infection	0	0	1	1
	Upper respiratory infection	0	0	2	2
	Urinary tract infection	0	0	1	1
Injury, poisoning, procedural complications	Fracture	0	0	1	1
Investigations	CD4 lymphocytes decreased	8	9	1	1
	Investigations - Other, specify	1	1	0	0
	Lymphocyte count decreased	11	13	0	0
	Neutrophil count decreased	2	3	1	1
	White blood cell decreased	4	5	0	0
Metabolism and nutrition disorders	Hypoglycemia	0	0	1	2
Musculoskeletal / connective tissue disorders	Pain in extremity	1	1	0	0
Nervous system disorders	Headache	3	9	0	0
	Nervous system disorders - Other, specify	0	0	1	1
Skin and subcutaneous tissue disorders	Rash maculo-papular	1	1	0	0
	Skin and subcutaneous tissue disorders - Other, specify	1	1	3	7
	Urticaria	0	0	2	2
Vascular disorders	Hypotension	1	1	0	0
	Phlebitis	1	1	0	0

In summary, clinical trial data from two independent studies in Stage 3 T1D have demonstrated efficacy in preserving C-peptide and lowering A1c in persons and suggest equal if not greater effects on metabolic responses in children compared to adults [6,61].

In the 2023 published case study, similar side effects were experienced in 6 pediatric participants who presented as at-risk for developing stage 3 T1D. CRS was reported in half of these participants and all had experienced serium sickness. No additional drug-related adverse events beyond CRS and SS were reported. 3 participants remained diabetes-free at 18 months, 3 years and 4 years respectively after treatment with ATG[2].

2.4 The unmet need for T1D therapies in children

Stage 3 T1D has its highest incidence in children ages 6 to 12 years, with a smaller peak later in adolescence. The hypothesis to be tested in the proposed clinical study is that ATG treatment will delay or prevent the diagnosis of Stage 3 diabetes in adults and children with high-risk, i.e. with Stage 2 diabetes. The immediate unmet clinical need for therapies that can delay or prevent Stage

3 T1D is emphasized by studies showing that even the most advanced insulin replacement therapeutic regimens have poor uptake in children with Stage 3 T1D[67]. Adolescents with diabetes also are the population that has the most sub-optimal glycemic control with mean HbA1c concentrations, even when under the care of endocrinologists, of > 9%[68]. Elevated HbA1c levels over time are associated with the risk of secondary end-organ complications including eye, kidney, and neurologic diseases[69]. The poor clinical outcomes of metabolic control and long duration of exposure to poor metabolic control are thought the be the basis for the observed loss of life expectancy of more than a decade, compared to non-diabetic peers, for individuals diagnosed with Stage 3 T1D as children[70]. Thus, the need for agents to delay and/or prevent progression from Stage 2 to Stage 3 T1D is most urgent for the pediatric population in order to reduce the time of exposure to poor metabolic control, a direct benefit to pediatric participants.

There are other immediate benefits of delay or prevention of Stage 3 diabetes for children and their families: These include avoiding daily repeated injections with insulin or use of mechanical devices, monitoring of glucose levels, constraints the diagnosis imposes on spontaneous activity. These quality-of-life aspects were recently highlighted during the FDA advisory panel meeting regarding the approval of teplizumab as a therapy to delay progression from Stage 2 to Stage 3 T1D (May 27, 2021). Any meaningful delay in the time to progression from Stage 2 to Stage 3 provides time for a child to and their families to better adapt to the lifestyle changes needed to cope with intensive insulin management. Likewise, advances in treatment of the disease including additional immunomodulatory interventions and automated insulin delivery are moving forward such that that delays in progression from Stage 2 to Stage 3 may permit persons with diabetes to take advantage of future therapies that are best suited for those who still retain some degree of endogenous beta cell function.

There are also important practical limitations in performing a prevention study with ATG that only enrolls adults. It would take many years to identify and enroll a sufficient number of adults to determine whether the treatment will alter progression from Stage 2 to Stage 3 T1D diagnosis – in the TN10 trial, previously completed by TrialNet, it took 1 year to enroll 6 people with diabetes over the age of 18. In addition, the time from Stage 2 to the diagnosis of Stage 3 diabetes is longer in adults compared to children, meaning it might take 5-8 years to detect a signal of meaningful delay in a study that was required to only enroll high-risk adults. Furthermore, participation in studies seeking to delay progression from Stage 2 to Stage 3 T1D is known to markedly reduce the incidence of diabetic ketoacidosis in children who do progress to Stage 3.

In summary, T1D is a disease that must be meaningfully studied in children to develop therapeutics capable of delaying progression from Stage 2 to Stage 3 disease. Preclinical studies support the ability of ATG to delay or prevent disease onset at a time when beta cell destruction is thought to be most active (i.e. Stage 2 diabetes in humans). In addition, both preclinical and clinical data have shown that ATG can slow the progression of autoimmune diabetes. The safety profile of low-dose ATG is well described with adverse event frequencies that are manageable and similar in children and adults. The disease course in adults is more protracted and the data from treating people with Stage 3 T1D with ATG suggest that the efficacy might be superior in children versus adults. Meanwhile children have the most urgent unmet need for agents that can delay or prevent Stage 3 diabetes because of their risk of secondary end-organ complications due to prolonged life exposure to dysglycemia, their observed shortened life expectancies, and the disease burden faced by people with T1D and their families. Recent experience in off-label treatment of ATG in pediatric participants at-risk of progression to Stage 3 T1D further demonstrates the tolerability of of ATG treatment in children as young as 5 years and the potential for low-dose ATG to meaningfully delay progression to Stage 3 T1D. As such, failing to enroll children in the proposed

trial would delay testing of ATG in this clinical setting and potentially deprive children of a treatment that may indeed be beneficial.

Given the totality of the available safety, efficacy, risk, and benefit data associated with low-dose ATG, and the fact that the Stages of T1D represent a continuum of the same disease process rather than different disease, statutory direct prospect of benefit requirements have been met for children to receive low-dose ATG as a potential means of preventing or delaying progression from Stage 2 to Stage 3 T1D.

3 STUDY DESIGN

3.1 Overview

This study is a 2-arm, multi-center, double blinded, 2:1 randomized, placebo-controlled clinical trial. The trial will investigate whether intervention with ATG will delay or prevent progression to Stage 3 T1D in individuals identified with a 2-year 50% risk for progression to Stage 3 T1D. A total of 101 people with 2-year 50% risk of progression to T1D will be enrolled. The treatment phase will consist of 2 infusions over 2-3 days. Participants will be followed up for at least 24 months from randomization. Participants who progress to stage 3 T1D will be followed to explore the treatment effect on C-peptide retention for at least one year.

3.1.1 Inclusion Criteria

Potential participants must **meet all** the following inclusion criteria:

1. Willing to provide informed consent or have a parent or legal guardian provide informed consent when the participant is <18 years of age.
2. Age ≥ 6 and < 35 years
3. At least two or more diabetes-related biochemical autoantibodies (mIAA, GADA, ICA, IA-2A, ZnT8A) present on the same sample. In the absence of other antibodies, ICA and GADA positivity alone will not suffice for eligibility in this trial.
4. Weight greater than the 5th percentile for age and sex.
5. BMI < 95th and > 5th percentile for age for those under age 18 years and < 30 and > 15 for adults (≥ 18)
6. ADA Stage 2 criteria* **AND** at least one of the following high-risk markers (occurring at the same visit) within 7 weeks (52 days) of randomization, defined below (for defining a 2-year 50% risk for progression to Stage 3 T1D):
    a. HbA1c ≥ 5.7 and <6.5%
    b. Index60 ≥ 1.4
        i. Index60 = 0.3695 × (log fasting C-peptide [ng/mL]) + 0.0165 × 60-min glucose (mg/dL) – 0.3644 × 60-min C-peptide (ng/mL)
    c. DPTRS ≥ 7.4

        DPTRS = (1.57 x log BMI) − (0.06 x age) + (0.81 x glucose sum from 30 to 120 min/100) − (0.85 x C-peptide sum from 30 to 120 min/10) + (0.48 x log fasting C-peptide)

    *Dysglycemia is defined as 2-hr glucose ≥ 140 and <200 mg/dL or fasting glucose ≥ 110 and <126 or 30, 60, or 90 minute glucose ≥ 200 mg/dL from OGTT[7].
7. CMV and/or EBV seronegative participants must be CMV and EBV PCR negative within 30 days of randomization and may not have had signs or symptoms of a CMV or EBV-compatible illness lasting longer than 7 days within 30 days of randomization.
8. CMV seropositive participants must be CMV PCR negative and all EBV seropositive participants must have EBV PCR < 2,000 IU/mL within 30 days of randomization and may

not have had signs or symptoms of a CMV or EBV-compatible illness lasting longer than 7 days within 30 days of randomization.

9. Seated blood pressure less than 130/80 mmHg for participants ≥ 18 years. For participants < 18 years seated blood pressure less than 95th percentile for age, sex and height.
10. Be at least 4 weeks from last live immunization.
11. Participants are required to receive non-live influenza vaccination at least 2 weeks prior to randomization when vaccine for the current or upcoming flu season is available.
12. Participants must have a negative COVID-19 test within 7 days of the first day of treatment if otherwise eligible.
13. Willingness to comply with study directed social distancing and protection from SARS-Cov-2 infection.
14. Be willing to forgo vaccines (other than killed influenza) during the 3 months after study drug treatment period (Days 0 and 1).
15. Be up to date on all recommended vaccinations based on age of participant*
16. With the exception of stage 2 T1D, participants must be healthy, as defined by absence of any other untreated diagnoses that the protocol committee deems to be a potential confounder.
17. If a female participant with reproductive potential, willing to avoid pregnancy (abstinence or adequate contraceptive method) through the completion of the study infusions and up to 3 months after study drug administration and undergo pregnancy testing prior to each study visit.
18. Must be residing or have accommodations within 1 hour of the infusion site during the two days of study drug infusions and must be within 1 hour of a medical care facility for 1 day after completion of infusion 2.
19. Participants must live in a location with rapid access to emergency medical services.

* Adult participants must be fully immunized. Pediatric participants who have not completed their primary vaccination schedule must receive all vaccinations allowable per the national/country-specific immunization guidelines for their current age prior to study drug delivery. Any remaining vaccinations should be given and continue per the schedule at least 3 months after study drug is administered. For COVID-19 vaccination, all participants will be strongly encouraged to be up-to-date with COVID-19 vaccine(s) as indicated by country-specific guidelines at least 2 weeks prior to randomization.

3.1.2 Exclusion Criteria

Potential participants must **not** meet any of the following exclusion criteria:

1. Immunodeficiency or clinically significant chronic lymphopenia: (Leukopenia (< 3,000 leukocytes /μL), neutropenia (<1,500 neutrophils/μL), lymphopenia (<800 lymphocytes/μL), thrombocytopenia (<100,000 platelets/μL).

2. Hemoglobin less than 13.5 g/dL for adult men and less than 12 g/dL for adult females and less than 11 g/dL for participants under age 18.

3. Active signs or symptoms of acute or chronic infection at the time of randomization including SARS-Cov-2.

4. Uncontrolled autoimmune thyroid disease and/or celiac disease (participants must be well controlled for the previous 6 months).

5. Evidence of prior or current tuberculosis infection as assessed interferon gamma release assay (QuantiFERON).

6. Currently pregnant or lactating or anticipate getting pregnant within the study period.

7. Require use of other immunosuppressive agents including chronic use of systemic steroids.

8. Evidence of current or past HIV or Hepatitis B or current Hepatitis C infection.

9. Any complicating medical issues or abnormal clinical laboratory results that may interfere with study conduct, or cause increased risk to include pre-existing cardiac disease, COPD, sickle cell disease, neurological disease, or blood count abnormalities.

10. A history of malignancies other than of skin.

11. Evidence of liver dysfunction with AST or ALT outside of the reference range.

12. Evidence of renal dysfunction with creatinine outside of the reference range.

13. Increased bilirubin (total and direct) outside of the normal limit (Participants with documentation of Gilbert's Disease permitted).

14. Vaccination with a live virus within the last 4 weeks.

15. Current or ongoing use of non-insulin pharmaceuticals that affect glycemic control within 7 days of screening.

16. Prior treatment with Teplizumab (either in a previous clinical trial or clinically).

17. Has participated in a clinical trial for diabetes prevention previously and received active study agent within 3 months of randomization.

18. Known allergy to ATG or any product excipient

19. Prior treatment with ATG or known allergy or anaphylactic reaction to rabbit-derived products or to any product excipient

20. Prior adverse reactions to heparin.

21. Any condition that in the investigator's opinion may adversely affect study participation will be reviewed by the Study Chair to ensure consistency and adjudicate whether or not the participant may compromise the study results

22. Any screening/baseline laboratory result not otherwise stated out of normal reference range and/or medical history that may increase the risk of the participant's participation in this trial.

23. Previously diagnosed with Stage 3 TID according to ADA criteria[7] *(see Appendix 3 for Criteria for diagnosis of diabetes)*

3.2 Description of Treatment Groups

This protocol will enroll at least 101 participants who will be randomly assigned to the following groups:

- 68 participants will be assigned to receive ATG
- 33 participants will be assigned to receive Placebo

3.3 Treatment Assignment and Double Masking

Participants who have provided consent and meet eligibility criteria will be randomized to one of the two arms. The randomization will be stratified by age (< 18 and ≥ 18). The participants will not be informed regarding the intervention assignment until the end of the study. The investigator and clinic personnel will also be masked as to study assignment. Laboratories performing assays for this protocol will be masked as to the identity of biological material to be studied.

3.4 Study Assessments

During the study, participants will frequently undergo assessments of their glucose tolerance status, insulin production, immunologic status, and overall health and well-being (see Schedule of Assessments). For participants who provide consent, samples will be drawn for storage in the National Institute for Diabetes and Digestive and Kidney Disease (NIDDK) Repository and at TrialNet Laboratory Sites for future analysis.

3.5 Quality Assurance

During the study, duplicate collections of blood samples for assays may be obtained in a small sample of participants for the purpose of quality surveillance of the performance of the central laboratories.

3.6 Post-Treatment Follow-up

All participants, regardless of T1D status will be followed for at least 24 months following the initial treatment visits of this trial (infusions 1 and 2) and at least 12 months following the study having reached the primary endpoint. Participants who have not developed Stage 3 T1D will be followed within this study for monitoring of glucose tolerance, measures of insulin production, and symptoms of T1D. Participants who develop Stage 3 T1D will also be followed within the context of this trial. These participants will complete an MMTT within 12 weeks of their date of diagnosis and will continue to be followed every six months after the diagnosis for at least 12 months or 12 months after the study has reached its primary end point, whichever is later.

4 PARTICIPANT MANAGEMENT

4.1 Overview

Eligible participants will receive either low-dose ATG or placebo. Infusion will be given as follows:

- Infusion 1 (visit 0) will be ATG/Placebo at 0.5 mg/kg IV over a minimum of 6 hours.
- Infusion 2 (visit 1) will be ATG/Placebo at 2 mg/kg IV over a minimum of 6 hours.

Participants will undergo screening visits to determine eligibility. Eligible participants will be randomized to one of two treatment groups (ATG or Placebo) as long they qualified for trial enrollment based on sections 3.1.1 and 3.1.2 for eligibility criteria. Study drug will be administered either in hospital (inpatient unit) or in outpatient infusion centers (criteria for outpatient defined later in this section) at the investigator's and institution's policy. Individuals will receive oral and IV premedication and the first dose of ATG/placebo will be administered over a minimum of 6 hours (maximum of 10 hours).

A CBC will be drawn prior to the first infusion and immediately after the completion of the first infusion. CBC will also be drawn prior to the 2nd infusion and the dose will remain at 2mg/kg, independent of the CBC results. The platelet count between the first 1st CBC and the CBC drawn prior to the second infusion will be reviewed to monitor for side effects of heparin-induce thrombocytopenia (criteria listed in section 4.5.2).

Participants will also have PT/INR/aPTT drawn prior to the first infusion and second infusion. Participants will also be asked about symptoms related to bleeding, bruising and nosebleeds prior to the start of the second infusion. A directed physical exam will be completed and vital signs will be collected from participant prior to the administration of infusion 2.

The second dose of ATG/placebo will be administered no less than 12 hours and no later than 30 hours from the start of the first infusion. The second dose of ATG/placebo will be given over a minimum of 6 hours (maximum of 10 hours).

Should a participant receive study agent as an outpatient (defined as leaving the medical facility in between administration of infusion 1 and infusion 2), the following criteria need to be met to allow for discharge from the facility:

- Resolution of any grade 2 adverse events the participant experienced during the infusion with or without medical intervention
- Clinically stable with normal mentation for at least 2 hours
- Stable vital signs without fever, hypotension or tachycardia for at least 2 hours
- Participant has been provided direct contact information for a study site investigator and access to emergency services should they become necessary
- Reside or have accommodations within 1 hour of the clinical trial facility

In the event that grade 2 or higher adverse events have not resolved by the end of the 2-hour observation period the study investigator will determine if the participant may be discharged to home or admitted to the hospital. Adverse events that would warrant admission to the hospital

include grade 3 events (including cytokine release syndrome) or other events as determined by the study investigator. If the patient is discharged, he/she will be contacted by the study investigator (must be a physician) at 4 hours after discharge from the infusion unit to assess adverse events. The participant/family will be provided contact information to reach the study physician at any time after discharge to discuss changes in health for clinical guidance. In the event that adverse events continue or worsen, or, in the judgement of the study investigator, warrant closer monitoring, the patient will be asked to return to the infusion center. The study investigator will make the determination whether patient should be admitted to the hospital for further observation and management.

All infusions are required to take place in a facility that has resuscitation capabilities.

4.2 Screening

After informed consent, participants will undergo assessments to determine if they meet eligibility criteria. Documentation of the participants understanding of the risks and benefits of the study will be collected through the Volunteer Understanding Assessment. Refer to Appendix 1 for assessments and procedures to be completed at the Screening visit. Rescreening will be permitted if the investigator considers it clinically appropriate.

4.3 Randomization and Treatment Masking

Eligible study participants will be randomized in a 2:1 allocation to either ATG or placebo treatment arms by the TrialNet Coordinating Center at the baseline visit once eligibility has been confirmed. Prior to randomization, participants will have a random glucose measurement to check their dysglycemic state has not progressed towards Stage 3 T1D. As long as this random glucose check is less than 200, randomization will proceed. If the value exceeds 200, the study physician will need to perform a safety check for Stage 3 T1D.

Randomization will be conducted using block randomization with stratification by whether the participant is less than 18 years of age or 18 years and older. Participants will be assigned a study randomization number corresponding to the treatment group assignment.

All participants and study personnel apart from the study pharmacist will be masked to the contents of the infusion. Treatment masking is inclusive of the ATG/Hydrocortisone/Heparin infusion or placebo infusion. Similarly, the premedication administration of methylprednisolone/placebo will also be masked. The only active therapeutics that all participants will receive regardless of randomization are the unmasked pre-infusion medications acetaminophen and diphenhydramine (a comparable antihistamine may be substituted for diphenhydramine in countries where it is unavailable).

4.4 Drug Administration

4.4.1 THYMOGLOBULIN (ATG)

THYMOGLOBULIN is indicated for prophylaxis and the treatment of acute rejection in patients receiving a kidney transplant. THYMOGLOBULIN is to be used in conjunction with concomitant immunosuppression. Each 10 mL vial contains 25 mg ATG (rabbit) as well as 50 mg glycine, 50 mg mannitol, and 10 mg sodium chloride.

4.4.2 ATG / Placebo Administration

Participants will receive study drug either in hospital or in outpatient infusion centers at the discretion of the investigator and institution where treatment will occur. Body weight at baseline (Time 0 – admission for the ATG/placebo infusion) will be used in calculating the doses for all infusions. Allowance in dose administration will be within +/- 5% of calculated dose.

To minimize the risk for thrombophlebitis associated with ATG infusion, 1000 units of heparin and 20 mg of hydrocortisone will be added to the ATG infusion bag for each dose given via peripheral intravenous administration. Investigators may choose to omit heparin from the prepared infusion solution in accordance with local policies for ATG administration. Since there is only a slight risk for thrombophlebitis for placebo infusion, the heparin and hydrocortisone will not be included in the infusion bag. Conversely, heparin may cause a change in the platelet count. The pre-infusion 1 platelet count will be compared with the pre-infusion 2 platelet count. If the pre-infusion 2 platelet count is within the normal reference range but more than 125,000 uL from the pre-infusion 1 platelet count, heparin will be withheld from the second dose of ATG. If the platelet count is less than 75,000 uL regardless of the difference between pre-infusion 1 platelet count and pre-infusion 2 platelet count, ATG/heparin infusion will be withheld.

The first dose (0.5mg/kg) will be infused over a minimum of 6 hours and the second dose (2mg/kg) over a minimum of 6 hours. The second dose will be given no less than 12 hours and no more than 30 hours from the start of the first infusion. The infusion can be slowed or temporarily stopped to treat signs or symptoms of cytokine release with a maximum infusion time for each infusion of 10 hours.

Information about participant's clinical signs and symptoms related to ATG infusion will be captured on infusion source documentation. Signs and symptoms assessed by the study clinician that are Grade 2 or more will be reported electronically to the TNCC. Signs and symptoms that are considered adverse events of special interest (see section 7.4) that are Grade 1 or more will be reported electronically to the TNCC.

4.5 Premedication

To reduce the risk of adverse reactions to ATG infusion for those assigned to active treatment, methylprednisolone 0.25 mg/kg IV will be given no less than 30 minutes before each infusion of active drug. Participants assigned to the placebo group will be given a placebo (saline) infusion similar in appearance no less than 30 minutes before each infusion.

Both groups: Participants in both groups will be pre-medicated with an antihistamine and acetaminophen PO at least 30 minutes before each infusion and every 4–6 hours as needed during the infusion, as follows:

- Diphenhydramine 1.25 mg/kg/dose to a maximum of 50 mg. *(Comparable antihistamine may be used in countries who do not have diphenhydramine available).*
- Acetaminophen 10–15 mg/kg/dose to a maximum of 650 mg.

4.5.1 Cytokine Release Syndrome

With ATG infusion, the participant may experience Cytokine Release Syndrome (CRS). The signs and symptoms can span a wide clinical spectrum.

Mild Reactions: For mild (grade 1) reactions per the NCI-CTCAE for CRS, the study medication will be continued. The investigator shall take one or more of the following actions, depending on the type of the reaction:

1. Administer additional doses of antihistamine and acetaminophen.
2. Reduce the rate of infusion by 50% or more.
3. For chills and rigors, meperidine may be considered.

Moderate Reactions: For moderate (grade 2) reactions per the NCI-CTCAE for CRS, the study medication may be interrupted at the investigator's discretion. The investigator shall take the following actions, depending on the type of the reaction:

1. Interrupt infusion if any of the following occurs:
   a. Oral temperature of > 40.0 °C
   b. Symptomatic bronchospasm or pulmonary edema
   c. Allergy-related edema
   d. Hypotension
2. When the temperature is < 38.5 °C and signs and symptoms improve, restart ATG.
3. Closely monitor the participant with pulse oximetry and a blood pressure monitoring; provide ongoing nursing evaluation until at least 2 hours after the infusion is completed.
4. If necessary, glucocorticoids can be given every 6 hours at a dose of 0.5 mg/kg of methylprednisolone or equivalent.
5. Additional supportive or resuscitative measures (such as the use of epinephrine) may be needed if clinically indicated.

Severe Reactions: For severe (grade 3) reactions or greater per the NCI-CTCAE for CRS, the study medication will be discontinued.

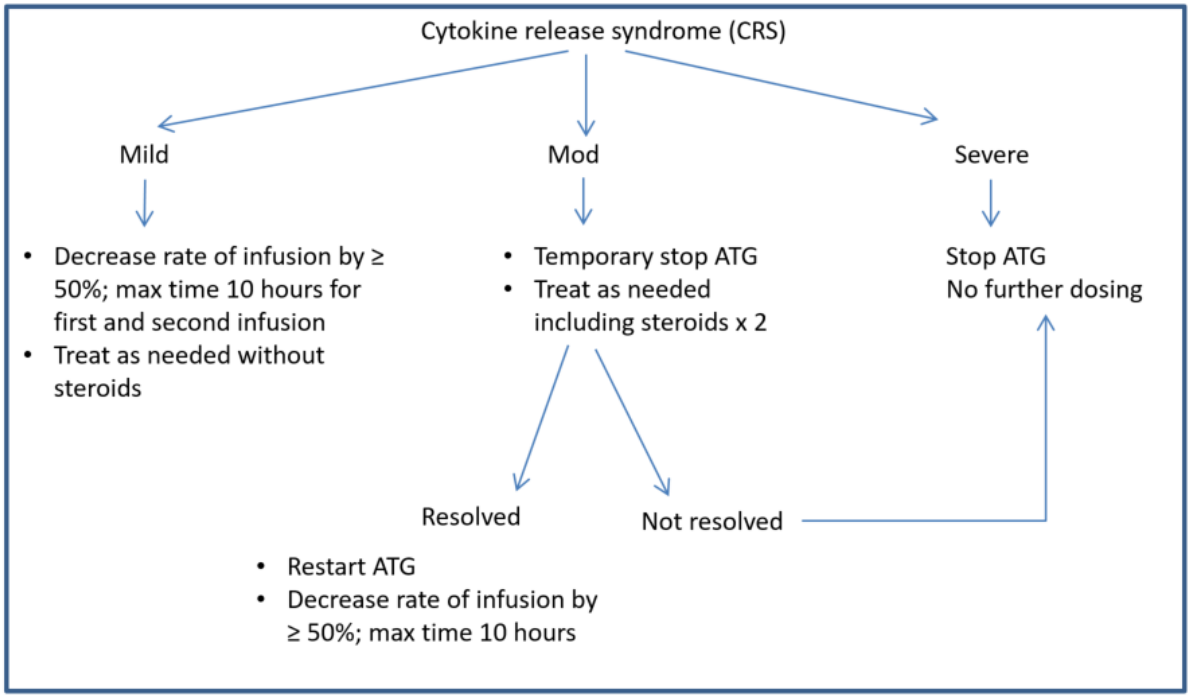

Figure 12. Rate Adjustments in Response to Cytokine Release Syndrome

4.5.2 Allergic Reactions

Hypersensitivity: In rare cases, people receiving ATG may experience hypersensitivity, which refers to immediate allergic, IgE mediated reactions to ATG. Such people primarily develop skin rash and respiratory distress early in the course of the infusion (usually within the first hour). For such reactions, the investigator shall take one or more of the following actions:

1. Discontinue the infusion.
2. Apply appropriate resuscitation measures, including administration of 0.3–0.5 mL aqueous epinephrine (1:1000 dilution) subcutaneously.
3. Use other resuscitative measures, as clinically indicated, including oxygen, intravenous fluids, antihistamines, corticosteroids, pressor amines, and airway management.

Mild to Moderate Reactions (Grade 1 and 2): For mild to moderate (grade 1 and 2) reactions per the NCI-CTCAE for allergic reactions, the study medication may be restarted at the discretion of the investigator.

Severe and Life-threatening Reactions: (Grade 3 and greater): For severe (grade 3) and life-threatening reactions (grade 4) per the NCI-CTCAE for allergic reactions, the study medication will be permanently discontinued.

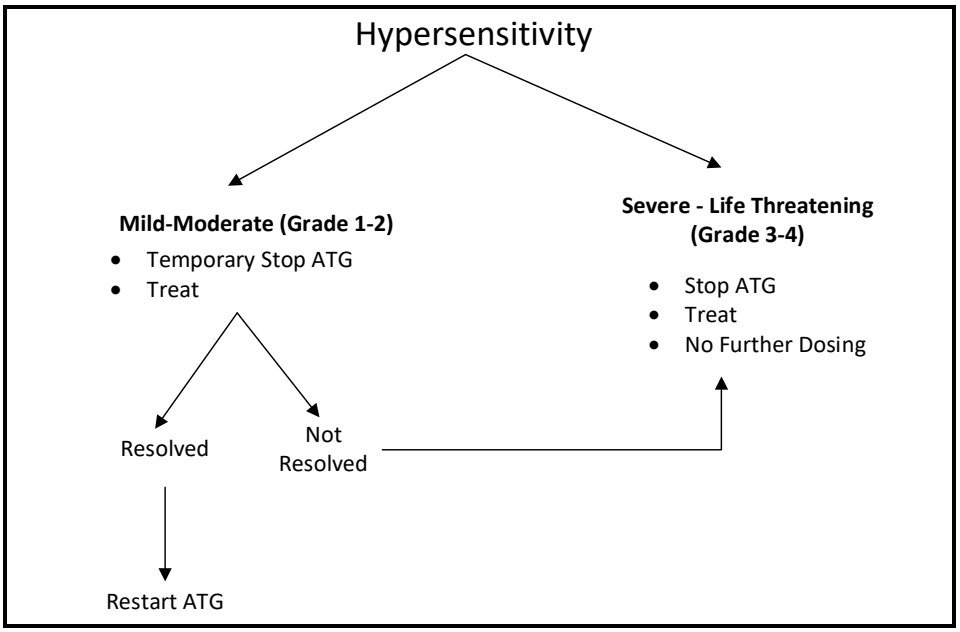

Figure 13. Rules for Temporary interruption or discontinuation of ATG due to Hypersensitivity Reaction

4.5.3 Serum Sickness

Serum sickness from host immunization against rabbit protein may occur 7–15 days after the first dose of ATG. Study participants may require glucocorticoid treatment for supportive care. The dose will depend on the severity of signs and symptoms. Site investigators should following the recommendations for prednisone administration (provided below) and contact the study chair for additional guidance regarding symptom management as needed for serum sickness.

For participants greater than 50kg, we recommend Prednisone as follows:

- Days 1-3: 50 mg every 12 hours
- Day 4: 40 mg every 12 hours
- Day 5: 30 mg every 12 hours
- Day 6: 20 mg every 12 hours
- Day 7: 10 mg every 12 hours

For participants less than 50kg we recommend Prednisone as follows:

- Days 1-3: 30mg every 12 hours
- Day 4: 20 mg every 12 hours
- Day 5: 10 mg every 12 hours
- Day 6: 5 mg every 12 hours
- Day 7: 5 mg once

Clinical judgment should be used in augmenting additional steroid therapy to provide the least amount of steroid required to provide symptomatic relief of serum sickness. Participants may refuse additional steroids but should be counselled that serum sickness symptoms may persist for 48-72 hours beyond the course that would be expected.

4.6 Concomitant Mediations

The use of concomitant medications will be assessed at each study visit and recorded on an appropriate source document and CRF. Participants will be asked to provide a full list of products used as each visit. This list should include any prescriptions, over the counter medications, dietary supplements or herbal preparations so investigators may review for potential interactions of any of the products in relation to the study agents.

Participants are required to receive killed (inactive) influenza vaccine for the current or upcoming season when available per local and national health guidelines. COVID-19 vaccination is strongly encouraged prior to study enrolllment to meet up-to-date vaccination status per country-specific guidelines. Date of all vaccinations will be documented as applicable.

Participants will be requested not to use any of the following medications during the study.

- Any anti-diabetic medication that influences insulin sensitivity or secretion.
- Vaccination with live vaccines from 4 weeks before enrollment to 3 months following last dose of study drug is not permitted. Non-live vaccines including influenza and COVID-19 are allowed during this time period.
- Any medication that may result in immunosuppression or immunomodulation.
- Systemic glucocorticoids (unless required during ATG administration or for the treatment of cytokine release syndrome or serum sickness).

Participants will be asked to avoid consuming biotin (also called vitamin B7) or biotin containing supplements in the 2-3 days prior to study visits where lab draws are required. In an FDA Safety Communication from November 2019, it was noted that biotin can interfere with certain lab test and cause incorrect results. Participants should discuss all supplements, including multivitamins with their study staff to check if it contains biotin and the amount contained in the product and discuss alternatives and/or a plan for stopping such medication prior to study visits.

If participants receive, or if the investigator believes that participants must receive, any of the above medications, the case must be discussed with the medical monitor and/or treating physician to determine if alternatives are available. The use of these medications must be documented on the source document and CRF.

4.7 Infectious Disease Screening

All participants will be strongly encouraged to be fully vaccinated for COVID-19 with an approved COVID-19 vaccine at least 2 weeks prior to enrollment. All participants will be asked to follow guidelines for social distancing and will be asked to wear a mask when outside of their home. Participants will be assessed for COVID-19 symptoms and close exposure to an individual diagnosed with COVID-19 at each study visit and before beginning study treatment with ATG/Placebo. Participants are required to be free of COVID-19 symptoms for 14 days prior to the screening visit and before the first infusion is administered. COVID-19 testing may be performed as required per local public health or institutional guidelines. A COVID-19 test is required to be done within 7 days of the enrollment visit (infusion 1). Point of care antigen test kits are acceptable.

Beyond that, no specific infectious diseases prophylaxis is warranted in this study. This decision is based on the limited duration and dose of exposure to ATG. In lieu of active serologic or virologic monitoring strategies, all participants will be counseled on an ongoing basis on the importance of notifying their research centers about the presence of signs or symptoms suggestive of infection especially over the 3 months after the infusion. They will also be counseled on the importance of notifying the research center about potential exposures to varicella, influenza, COVID-19 or other

infectious illnesses. In addition, during the first 2 weeks post ATG infusion, all research participants will be contacted every other day and queried about the presence of signs or symptoms (e.g. fever, rhinorrhea, cough, sore throat, mouth sores, myalgia, arthralgia, etc.) that might represent markers of active infection or serum sickness. From 2 weeks post infusion to 3 months after the infusion, participants will be contacted weekly to monitor for symptoms of infection and/or illness. Specific algorithm(s) for the evaluation and management of those study participants identified as having signs and/or symptoms concerning for infection are provided in the manual of operations for this study and are directed based on the participant's specific symptomatology. This will provide general and, in some cases, specific recommendations for the assessment of study participants.

All participants will have CMV and EBV serology determined prior to study enrollment. Participants who are CMV and/or EBV seronegative must be CMV and/or EBV PCR negative within 30 days of randomization. Participants who are CMV seropositive at screening must have a negative CMV PCR and those who are EBV seropositive must have an EBV PCR <2,000 IU/mL within 30 days of randomization. All participants may not have had signs or symptoms of a CMV or EBV compatible illness lasting longer than 7 days within 30 days of randomization. Additional CMV and EBV or other infectious disease monitoring will be done only if clinically indicated.

4.8 Diabetes Management for Participants Progressing to Stage 3 T1D

Once the participant progresses to Stage 3 T1D, the participant is to receive management of their diabetes per current standards of clinical care. The goal of the treatment will be to keep the HbA1c levels within the currently recommended American Diabetes Association target range in the absence of clinically significant or severe hypoglycemia or diabetic ketoacidosis. The primary responsibility for diabetes management will be on the treating or referring diabetes care provider, but the research study team will provide additional support through regular interaction. Records of communication with the participant will provide source documentation of this interaction. Participants will not be permitted to use non-insulin pharmaceuticals for glycemic control. Insulin infusion pumps and hybrid closed loop automatic insulin delivery systems are permitted.

The Safety Monitoring Committee will evaluate individual HbA1c data outside these ranges, and will provide additional guidance to the clinical site as needed to bring glycemic control within goals. Any episode of severe hypoglycemia will be promptly reviewed by the Medical Monitor with recommendations for changes in diabetes management, if any, conveyed to the clinical site in conjunction with the Safety Monitoring Committee.

5 STUDY ASSESSMENTS

See Appendix 1 for detailed schedule of assessments

5.1 General Assessments

Study visits for all groups will occur according to the Schedule of Assessments. General assessments include:

- Medical history
- Participant experience assessments and patient reported outcome measures (i.e., quality of life, treatment satisfaction, etc.)
- Complete or directed physical exam (including Tanner Stage assessment)
- Vital Signs
- Concomitant medications
- Adverse events
- Diabetes Management (for those who progress to Stage 3)

5.2 Laboratory Assessments

The following general laboratory assessments will be performed during the study, as described in the Schedule of Assessments:

- Chemistry (sodium, potassium, chloride, CO2, glucose, BUN, creatinine)
- Liver function tests (ALT, AST, LDH, alkaline phosphatase, total protein, albumin, total and direct bilirubin)
- Hematology (complete blood count with differential and platelets)
- Prothrombin time (PT), activated partial thromboplastin time (PTT), International Normalization Ratio (INR)
- CD4/CD8 panel
- Interferon-Gamma Release Assay Test (IGRA) *for Tuberculosis testing*
- Diabetes Autoantibodies
- Antibodies to HIV, hepatitis B (antiHBcAb, HBsAg), hepatitis C (HCV), Cytomegalovirus (CMV IgG, IgM), and Epstein-Barr Virus (EBV IgG, IgM, EBNA)
- CMV and EBV PCR
- COVID testing within 7 days of dosing (infusion 1) *Additional Covid-19 testing will be done as needed or at investigator's recommendation.*

Additional assessments include:

- Pregnancy test before ATG/Placebo infusion #1 and all study visits post-treatment for all women of reproductive age.
- COVID-19 Assessment Form

5.2.1 Pharmacokinetic and Immunogenicity Assessments

Blood samples will be obtained from a subset of participants at the timepoints indicated in the schedule of assessments (Appendix 1) to evaluate pharmacokinetic and

immunogenicity data. Pharmacokinetic assessments of the samples will include concentration-time data of ATG to obtain primary PK parameters, clearance and volume of distribution. Immunogenicity assessment will include anti-drug antibodies. Assessment of both pharmacokinetic and immunogenicity samples will be performed by a laboratory appointed by TrialNet and be analyzed using validated, specific and sensitive immunoassay methods. HLA Typing will also be performed. Samples will be collected from 12 participants in each of the following age groups: <18 years and ≥18 years at randomization.

5.3 Mechanistic Outcome Assessments

TrialNet will perform immune and genetic assays to further understand mechanisms that may be underlying the type 1 diabetes disease process and response to therapy. TrialNet proposes to evaluate the relationships between changes in immune cells and the delay in onset of T1D. Transcriptomic and serologic studies are planned. In addition, the relationship between specific genotypes and the responses to ATG may be explored. For these purposes, samples for PMBC, DNA, RNA, plasma, and serum may be obtained. The following HLA typing will also be performed: Class I (HLA-A, B, and C) and Class II (DRB, DQA, DQB).

Fecal samples will also be collected from willing participants to explore whether the gut microbiome can predict response to therapy and whether therapy induces changes in the microbiome.

5.4 Metabolic Outcome Assessments

Metabolic assessments will consist of:

- Oral Glucose Tolerance Test (OGTT)
- HbA1c
- 7-10-day blinded CGM wear following screening and all OGTT visits
- 7-10 day unblinded CGM wear following MMTT visits (for participants who progress to Stage 3 T1D)
- Mixed Meal Tolerance Test (MMTT) using BOOST® High Protein Drink (to be completed by participants who progress to Stage 3)

5.4.1 OGTT Frequency and Monitoring of Symptoms of Diabetes Progression

OGTTs will be done at month 3, 6, and then every 6 months thereafter. The study staff will contact the participants every 3 months for a monitoring call, and will obtain any history of signs or symptoms (e.g. polyuria, polydipsia, weight loss, etc) of diabetes or the finding of any random glucose > 200 mg/dl, or the diagnosis of T1D by their primary care provider. Participants will be asked to contact the study staff in-between any study visit in the event that these occur. In the event that they occur, the participant will be asked to undergo an OGTT as soon as possible and prior to the next scheduled study visit or monitoring call. As indicated in the SOA (Appendix 1), OGTTs may be performed as needed between scheduled study visits.

5.4.2 Blinded Continuous Glucose Monitoring

The participant will be instructed to wear the device continuously for 7-10 days following the screening visit and all visits with an OGTT. The participant will be masked to the blood glucose readings and will be instructed to carry on with normal daily diet and activity habits (CGM readings unmasked to participant if they progress to Stage 3 T1D during the study). Once the 7-10-day wear period has ended, participants will then mail the device back to the site for appropriate data download. See Appendix 1 for timepoints blinded CGM wear is required.

5.4.3 Unblinded Continuous Glucose Monitoring

If a participant progresses to Stage 3 T1D, the participant will continue study visits according to Appendix 2. Following all visits with an MMTT performed, the participant will be instructed to wear an unblinded CGM device continuously for 7-10 days. If a participant is wearing a CGM as part of their clinical care, the participant may opt out of using the study provided CGM device and the site will ask the participant if they would be willing to provide CGM data from the 7-10 days range per protocol.

5.5 Visit Windows

Randomization and the baseline visit must occur within 7 weeks (52 days) of having met two or more of the high-risk markers. All subsequent treatment visits and follow up visits in Appendix 1 must occur within the time limits specified below:

	Visit Windows
Visit -1:	Open window for screening for any participants with 2+ Antibodies
Visit 0:	Randomization within 7 weeks (52 days) of having met ADA Stage 2 criteria and at least one of the high-risk markers. First dose of ATG/placebo given over minimum of 6 hours and maximum of 10 hours. The infusion can be slowed or temporarily stopped to treat signs or symptoms of cytokine release with a maximum infusion time for each infusion of 10 hours.
Visit 1:	The second dose should be given no earlier than 12 hours and no later than 30 hours from the start of the first infusion. The second infusion of ATG/placebo given over a minimum of 6 hours and maximum of 10 hours. The infusion can be slowed or temporarily stopped to treat signs or symptoms of cytokine release with a maximum infusion time for each infusion of 10 hours.
Visits 1.1-1.3*	PK samples should be drawn on target days 2 and 4. Window for day 7 PK draw is ±1 day.
Visit 2:	±3 days

Visits 3:	±7 days
Visits 4 to 13:	±14 days

*subset of participants, as detailed in section 5.2.1.

5.5.1 Visit Window for Participants who Progress to Stage 3

Once a participant has reached primary endpoint (Stage 3 T1D), the participant will continue study visits according to Appendix 2. The initial study visit is to take place within 12 weeks from date of diagnosis. Study visits will then be completed every six months and within +/- 14 days from the target date. The target date will be calculated from the date of the participant's initial visit post diagnosis of stage 3 T1D (1st MMTT visit). Participants who develop diabetes will be followed for a minimum of 1 year. Once follow-up within this study has finished, participants have the option to be followed in TrialNet's LIFT protocol for monitoring or other studies that are available.

5.6 Withdrawal from treatment

The study will be conducted according to the intent-to-treat principle. Once randomized into the study, a participant will be expected to undergo all scheduled follow-up assessments and will remain within the assigned treatment group for purposes of statistical analysis regardless of the actual course of treatment administered. Withdrawal from treatment does not automatically entail withdrawal from the study. While the study is actively recruiting and participant visits are conducted per Appendix 1 and 2, withdrawal from the study will only occur if the participant dies or withdraws consent. Participants who withdraw consent are classified as inactive but may again become active upon re-entry into the study, if they so choose.

Withdrawal from treatment can occur for a number of reasons, some of which are outlined below.

A participant may elect to discontinue study medications, may be unable to continue them, or may be withdrawn (temporarily or permanently) at the discretion of the Principal Investigator if s/he determines that it is unsafe to continue or there is a significant change in the risk/benefit.

Non-pregnant individuals who are withdrawn from treatment should remain in the study and undergo scheduled assessment visits as appropriate and any additional visits as needed to address ongoing AEs. OGTT tests will not occur while an individual is pregnant.

5.7 Re-Entry into the Study

In some circumstances, a participant may temporarily discontinue study participation and then later, could resume follow-up visits. If the participant decides to return for study follow-up assessments at a later date, he or she will be allowed and encouraged to do so.

5.8 Interruption of Enrollment/Trial Cessation

This section lists clinical and laboratory adverse events that will necessitate interruption of enrollment in the trial as a whole. As part of their ongoing safety review, the DSMB will make independent judgments regarding other adverse events requiring trial interruption. In addition, should the study be stopped, the FDA will be informed and the study will not resume until both the DSMB and the FDA are in agreement.

1. Any drug related death or Grade 4 SAE; During this trial, any grade 4 SAE death event will be temporarily considered unexpected and potentially drug-related until the event is reported and reviewed by the FDA and DSMB. In this event, the trial will be interrupted, including dosing of participants already enrolled and enrollment of new participants, until the grade 4 SAE or death event is reviewed by both the FDA and DSMB and both agree to the restart of trial enrollment and study agent administration.
2. Severe adverse event (defined in section 7.3) that is possibly, probably or definitely related to study treatment in any of the first 3 participants, or severe adverse events in the same organ system in any 2 participants with the exception of Grade 3 lymphopenia within the first 30 days of drug treatment. This criterion supersedes all rules below (3-8).
3. Occurrence of anaphylaxis during study treatment in any participant. Anaphylaxis is a serious allergic reaction that is rapid in onset and may cause death. Anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled:
    1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (e.g., generalized hives, pruritus or flushing, swollen lips-tongue-uvula)

        AND AT LEAST ONE OF THE FOLLOWING:

        i. Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reduced peak expiratory flow (PEF), hypoxemia)

        ii. Reduced Blood Pressure (BP) or associated symptoms of end-organ dysfunction (e.g., hypotonia [collapse], syncope, incontinence)

    2. Two or more of the following that occur rapidly after exposure to a likely allergen for that person (minutes to several hours):
        1. Involvement of the skin-mucosal tissue (e.g., generalized hives, itch-flush, swollen lips-tongue-uvula)
        2. Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
        3. Reduced BP or associated symptoms (e.g., hypotonia [collapse], syncope, incontinence)
        4. Persistent gastrointestinal symptoms (e.g., crampy abdominal pain, vomiting)

3. Reduced BP after exposure to known allergen for that person (minutes to several hours):
    i. Infants and children: low systolic BP (age specific) or greater than 30% decrease in systolic BP*
    ii. Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person's baseline

*Low systolic blood pressure for children is defined as less than 70mmHg from 1 month to 1 year, less than (70 mmHg + [2x age]) from 1 to 10 years, and less than 90 mmHg from 11 to 17 years.

4. Any grade 3 cytokine release syndrome (according to CTCAE criteria) that does not resolve within 72 hours or grade 2 cytokine release syndrome that does not resolve within 48 hours at any time in 2 patients.
5. The occurrence of ALT or AST ≥ 3x ULN and bilirubin > 2x ULN at any time in any one of the first 10 participants more than 2 of any ATG treated participant, with the exception of those who have been diagnosed with Gilbert's syndrome prior to randomization and have met the criteria listed in sections 4.5.1, 4.5.2 or 5.6 for this occurrence.
6. Grade 3 hypotension or any urgent care visit and/or hospitalization with or without cytokine release syndrome or anaphylaxis any time in 1 of the first 10 or more than 2 of the total ATG treated participants.
7. Grade 2 thrombocytopenia (i.e. < 75,000/uL) at any time in more than 2 of the ATG treated participants.
8. Clinical mononucleosis syndrome in a patient who is EBV seropositive at the time of enrollment, between 5 days and 6 months after the last dose of study drug, or at a later time if designated as probably or possibly related to study drug by the study investigator, in more than 2 patients. The syndrome may include: Grade 2 or above fever, pharyngitis, lymphadenopathy, splenomegaly, or rash, with detectable EBV viral load

6 PARTICIPANT SAFETY

6.1 Risk, Benefits and Inclusion of Children

The risks of this study are presented in this protocol and in the informed consent form. This study will examine whether ATG will delay or prevent the onset of T1D. As discussed above, there is prospect of direct benefit for participants who are children by participation in this study.

Moreover, our experience in using ATG in people with Stage 3 disease has shown a similar safety profile in adults and children and supports a favorable risk-benefit ratio in children with Stage 2 T1D as discussed above. Assent of children along with consent of the parents or legal guardians will be obtained prior to any study procedures. This research proposal in children is therefore consistent with United States Department of Health and Human Services, Protection of Human Participants, subpart D, section 46.405 (research involving greater than minimal risk but presenting the prospect of direct benefit to individual participants) and with Subpart D. 50.52 (Clinical investigations involving greater than minimal risk but presenting the prospect of direct benefit to individual participants).

6.2 Potential Risks

6.2.1 ATG

ATG has been widely used in transplantation and autoimmune disorders[47]. In the ITN START study, participants received ATG at a total dose of 6.5 mg/kg, a dose which has been effective and well tolerated in bone marrow and solid organ transplantation, and well below the dose of 20 mg/kg that has been used for some transplantation protocols. For this study, the doses will be administered as 0.5 mg/kg during the first infusion and 2 mg/kg during the second infusion to reduce adverse reactions. ATG will be given over at least 6 hours for both infusions. Participants will receive pre-medication with acetaminophen and diphenhydramine (or equivalent) and participants randomized to active therapy will receive methylprednisolone prior to the in the infusion that contains ATG. This is to minimize side effects from possible cytokine release syndrome (CRS) Participants will be closely observed in the hospital/outpatient setting during the treatment period and will be contacted between treatments for assessment of adverse events and management should they occur. Symptoms of CRS may include fever, chills, rigors, headache, tremor, nausea, vomiting, diarrhea, abdominal pain, muscle and joint pain, and malaise. Anaphylactic reactions have been rarely reported with ATG. Delayed reactions such as serum sickness occur in approximately ¾ of actively treated participants between 9-14 days post-infusion. Thrombophlebitis may also develop following infusion with ATG.

ATG contains a variety of antibodies that may cross-react with cell-surface markers. ATG causes lymphocyte depletion with marked depletion of lymphocytes occurring acutely. The circulating number of T cells increases with cessation of therapy, usually reaching pre-treatment levels of CD8+ T cells by 2 months. Participants are at low absolute but relatively increased risk for opportunistic infection during this window of recovery and will receive close surveillance for primary viral infections, viral reactivations, and bacterial infections[71,72].

ATG may also lead to leukopenia and thrombocytopenia. Effects are dose-dependent and are mainly encountered with over dosage. In up to 3% severe thrombocytopenia may occur but this is invariably seen in doses 4-5 times that which we are proposing. Thrombocytopenia in these participants often occurred in a postoperative transplant setting and with other immunosuppressants.

Transient abnormalities in liver function tests have been described in people with aplastic anemia treated with ATG preparations. Such adverse events have not been noted in numerous other clinical settings in which ATG has been used, and it is not clear if this is related to the underlying disease or to associated medications used.

Conflicting data exists about the risk of EBV-related lymphoproliferative disease. Furthermore, in people with renal transplant, the overall risk is low (0.25%-0.85%) even though these populations received multiple immunosuppressive therapies and were on continuous immunosuppression (30). There were no reported cases of EBV-associated lymphoproliferative disease in over 1,675 treated participants (including children) treated for aplastic anemia.

Treatment with ATG may increase the risk for serum sickness and may preclude future exposure to rabbit-derived immunotherapeutics including second courses of ATG.

There is the possibility that, instead of delaying or preventing the development of stage 3 type 1 diabetes (clinical diagnosis), ATG could increase the destruction of islet cells in the pancreas. The risk of this happening is unknown. This could cause type 1 diabetes to develop more quickly.

6.2.2 Insertion and Wearing a Continuous Glucose Monitor (CGM)

Inserting and wearing a CGM device may cause mild pain, discomfort, inflammation or irritation from the device, which could be related to the insertion needle or to adhesives and tapes used to secure the CGM device to the skin. Minor risk of infection at the needle insertion site is also possible. The CGM device will be inserted by the investigator or trained staff using techniques designed to minimize the risk of inflammation or infection. Signs and symptoms of skin problems and site infections are to be discussed with each participant.

6.3 COVID-19

ATG is not believed to increase the risk of acquiring COVID-19. However, individuals who have received ATG may be at risk for a more severe clinical course with SARS-CoV2. To mitigate this risk the following precautions will be instituted:

1) Those at highest risk for severe COVID-19 infection are excluded from enrollment.

2) All participants will be advised to follow country-specific guidelines and recommendations regarding social distancing, masking, and avoiding contact with individuals with COVID-19 within 10 days, crowded spaces for immune compromised individuals.

3) Although no live vaccinations will be allowed during this trial, other non-live vaccines such as COVID-19 and influenza are allowed; however, the effectiveness of non-live vaccinations given in the 3 months following the study treatment phase is unknown. Participants are strongly encouraged to be up-to-date on COVID-19 vaccine(s) at least 2 weeks prior to randomization (follow country-specific/regional guidelines). Samples to evaluate response to vaccination may be

collected in such participants. In addition, if future recommendations are issued by the national health governing body regarding COVID-19 vaccinations, the participants will be encouraged to follow them.

All coronavirus-related events are to be reported as adverse events. If the event meets the criteria of a SAE, expedited SAE reporting is to be followed and such events should be reported to the sponsor within timelines.

6.4 Pregnancy

Female participants with reproductive potential will be instructed to use effective means of birth control (which includes abstinence) from randomization until 3 months post second infusion of study drug to assure safety. They will also be asked to avoid pregnancy until the conclusion of the study. This is to assure accurate endpoint measurements. They will undergo urine pregnancy testing at the start of every study visit. All pregnancies that are identified during the study must be followed to conclusion and the outcome of each must be reported. The investigator should be informed immediately of any pregnancy occurring in a female participant. Monitoring of the participant should continue until the conclusion of the pregnancy. Participants that are found to be pregnant while on this study will still be followed for safety and other study measures as appropriate.

All pregnancies within the trial (either the participant female or the participant female partner) should be reported to the external sponsor and Sanofi using the relevant Pregnancy Reporting Form within 24 hours of notification.

Pregnancy is not considered an AE unless a negative or consequential outcome is recorded for the mother or child/fetus. If the outcome meets the serious criteria, this would be considered an SAE (any complication of pregnancy should be reported as a SAE if meeting seriousness criteria: spontaneous abortion (even if no hospitalization), and congenital anomalies.)

Women who required insulin during or shortly after pregnancy but for who, insulin requirements resolved post-partum will not be considered to have previously had stage 3 T1D and will be eligible if all other study inclusion/exclusion criteria are met.

6.5 Protecting Against or Minimizing Potential Treatment Risks

Participants will not be enrolled who have other active serious medical problems. Frequent monitoring with history, physical examination, and laboratory studies will allow for early identification of adverse events. All participants will be required to have adequate hemoglobin to allow safe frequent venipuncture. Every attempt will be made to minimize the number of venipunctures.

All study drug infusions will take place in a facility that has resuscitation capabilities, and participants will be closely monitored during and after the treatment.

Participants will be counseled about the potential risk for infections and the need to report any change in health status between or at the time of visits. Directed questioning about concurrent illness will occur before each infusion. No infusion will occur in those with signs or symptoms indicative of active infection.

7 ADVERSE EVENT REPORTING AND SAFETY MONITORING

7.1 Adverse Event Definition

In this clinical trial, an adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom or disease whether or not associated with the treatment and study procedures.

Throughout the study, the investigator must record all adverse events on source documents. Adverse Events of Special Interest (AESIs) as described in section 7.4 which are Grade 1 or greater per the NCI CTCAE must be reported electronically to the TNCC. Events not related hypoglycemia, or hyperglycemia which are Grade 2 or greater per the NCI CTCAE (see Section 7.6 and 7.7 below) must be reported to TNCC as AE. The investigator should treat participants with adverse events appropriately and observe them at suitable intervals until the events resolve or stabilize.

Adverse events may be discovered through:

- observation of the participant;
- questioning the participant;
- unsolicited complaint by the participant.

Questioning of the participant should be conducted in an objective manner.

7.2 Adverse Reaction

An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse events for which there is reason to conclude that the drug caused the event. Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction, which means any adverse event caused by a drug. Examples of evidence that suggest a causal relationship (reasonable possibility) between the drug and the adverse event include:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the populations exposed to the drug
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

7.3 Serious Adverse Event/Reaction

A serious adverse event (SAE) or reaction is defined as "any adverse event occurring at any dose that suggests a significant hazard, contraindication, side effect, or precaution." An adverse event

or suspected adverse reaction is considered “serious” if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

1. Death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy must be reported whether it is considered to be treatment related or not.
2. A life-threatening adverse event. The term life-threatening is defined as (1) diseases or conditions where the likelihood of death is high unless the course of the disease is interrupted and (2) diseases or conditions with potentially fatal outcomes, where the endpoint of clinical trial analysis is survival (21 CFR 312.81(a)).
3. Inpatient hospitalization or prolongation of existing hospitalization with the exception of hospitalization relating to initial diagnosis of stage 3 type 1 diabetes. Symptoms/reactions of stage 3 T1D onset which require intravenous intervention for diabetes management in an emergency room, physician’s office or visiting nurse.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

Adverse event or suspected adverse reaction are considered “life-threatening” if they are (1) diseases or conditions where the likelihood of death is high unless the course of the disease is interrupted and (2) diseases or conditions with potentially fatal outcomes, where the endpoint of clinical trial analysis is survival (21 CFR 312.81(a)).

Regardless of the relationship of the adverse event to study drug, the event must be reported as a serious adverse event if it meets any of the above definitions.

7.4 Adverse Events of Special Interest

An adverse event of special interest (AESI) can be serious or non-serious but is one of concern specific to the sponsor’s product. Adverse events categorized as such require ongoing monitoring and rapid communication by the investigator to the sponsor. AESIs grade 1 or higher will be reported electronically to the TNCC. The DSMB and the FDA will be notified of Grade 3 or greater or hospitalization events in relation to Serum Sickness. AESI include the following conditions/symptoms:

- Anaphylaxis
- Cytokine Release Syndrome
- Hematologic Effects (i.e. lymphopenia. Neutropenia, thrombocytopenia)
- Serum Sickness
- Infections
-

7.5 Unexpected Adverse Event

An adverse event/reaction is considered unexpected when the nature (specificity) or severity of the event is not consistent with the risks described in the Investigator's Brochure or the informed consent document. Unexpected refers to an experience that has not been previously observed. This includes events that occur more frequently than expected.

7.6 Grading Event Severity and Causality

TrialNet has adopted usage of the National Cancer Institute (NCI) Common Technology Criteria for Adverse Events (CTCAE) and/or study-specific criteria for classification to describe the severity of adverse events with the exception of severe hypoglycemia and hyperglycemia. Hypoglycemia and hyperglycemia will be reported as adverse events only in the case of requiring the assistance of others due to loss of consciousness or diabetic ketoacidosis. TrialNet Investigators will also provide an assessment of relationship of AE to study drug as not, unlikely, possibly, probably, or definitely related.

The reference safety information to be used by the TrialNet Investigators for evaluation of expectedness of adverse events shall be the adverse reaction section of the current approved product label available in the country.

7.7 Adverse Event Reporting and Monitoring

The investigator will grade their severity according to common toxicity criteria or study-specific criteria and will decide of their relation to therapy. Events will be assessed and reported consistent with the ICH Guidelines for Good Clinical Practice, 21 CFR 312.32 for expedited safety reporting, and per the guidance of the DHHS Office for Human Research Protections (OHRP). General descriptions of grading are as follows:

- Grade 1 are mild adverse events requiring no specific medical intervention or asymptomatic laboratory findings
- Grade 2 are moderate adverse events, minimal, local, non-invasive intervention
- Grade 3 are severe and undesirable events, significant symptoms requiring hospitalization or invasive intervention
- Grade 4 are life-threatening or disabling events
- Grade 5 are fatal events (death)

The adverse event case report form for the protocol must be completed for all adverse events assessed as grade 2 or higher and grade 1 and higher for AESIs. The investigator is responsible for regular participant follow-up and for ensuring all adverse events are documented and reported appropriately. For reporting serious adverse events (SAE), in addition to the AE case report form, the site should also complete a MedWatch Form and email/fax to the TNCC *within 24 hours of when the site is notified of the event.* This will be reviewed by the TrialNet Medical Monitor and the DSMB as appropriate. Death must be reported immediately. Event outcome and other follow-up information regarding the treatment and resolution of the event will be obtained and reported when available, if not known at the time the event is initially reported. The follow-up information should

contain sufficient detail to allow for a complete medical assessment of the case and an independent determination of possible causality.

Data will be submitted to the TNCC and be kept by TNCC in keeping with all applicable agreements and when requested, such as for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study reports. The investigators will ensure prompt reporting of all events to the TNCC who will facilitate the required safety reporting to any oversight bodies as applicable (e.g., Medical Monitor, DSMB, cIRB, sponsor). The investigator must continue to follow the participant until the AE has subsided or until the condition becomes chronic in nature, stabilizes (in the case of persistent impairment), or the participant dies. Upon the receipt of follow-up information, the investigator must update the AE case report form and the MedWatch Form, if applicable.

Unless otherwise specified, all SAEs (regardless of relationship to the investigational agents) must be reported to the TNCC no later than 24 hours of learning of the event. The Medical Monitor's assessment and determination of the nature of the SAE (seriousness, expectedness, relatedness) and determination of expedited reporting mechanism (e.g. for SUSAR) to the FDA will be kept at TNCC as part the of safety monitoring record. The TNCC is responsible for immediately notifying the sponsor when a SUSAR is identified in order to comply with FDA reporting requirements. Any additional follow-up information obtained from the site after the initial report should be submitted to the TNCC via an updated MedWatch form as applicable. The TNCC will ensure the sponsor is notified of any follow-up MedWatch forms received in compliance with all reporting guidelines.

Adverse events will be assessed by the TrialNet Medical Monitor. The DSMB will conduct regular safety reviews approximately every three to six months (and otherwise as needed) of adverse events by treatment group assignment. Serious adverse events as well as adverse events leading to study discontinuation will be reviewed by the DSMB.

For SAEs that are unexpected and considered possibly or probably drug related, the TNCC can provide information on frequency of similar events, and generate FDA form 3500A reports (MedWatch form) for distribution to FDA, NIDDK, DSMB and site investigators. Expedited safety reports will be submitted to the FDA by the sponsor, NIDDK per the required reporting timelines. The TNCC will also be primarily responsible for sending required safety information to Sanofi.

TrialNet will inform Sanofi of all SAEs and Adverse Events of Special Interest (AESIs) regardless of Investigator's causality within 1 business day.

8 STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

Analyses of study data will be conducted to address all objectives of the trial and other interrelationships among data elements of interest to the investigators and of relevance to the objectives of the study. Analyses by gender and race/ethnicity, as appropriate, are also planned.

Primary analysis of treatment effect will be conducted under the intention-to-treat principle whereby outcome data from all participants randomized will be included regardless of treatment compliance.

8.1 Primary Outcome and Analyses

The primary outcome is the elapsed time from random treatment assignment to the development of diabetes or time of last contact among those randomized.

Criteria for stage 3 diabetes onset are, as defined by the American Diabetes Association (ADA), based on glucose testing, or the presence of unequivocal hyperglycemia with acute metabolic decompensation (diabetic ketoacidosis) as defined below:

1. Unequivocally symptomatic* AND at least one of the following:
    1. Random glucose ≥ 200 mg/dl [11.1 mmol/L] OR
    2. Fasting glucose ≥ 126 mg/dl [7 mmol/L] OR
    3. OGTT = diabetic OR
    4. HbA1c ≥ 6.5%

    * Unequivocal symptoms: severe/persistent polyuria, polydipsia and/or significant unexplained weight loss

2. If not unequivocally symptomatic, two separate objective measures are required for diagnosis. These tests must occur at least one day apart but as soon as possible and must be consecutive, defined as without an intervening non-diabetic OGTT.
    1. The preferred object measures for diagnosis are:
        1. 2 diabetic OGTTs, not on the same day (the confirmatory OGTT is the date of diagnosis)
        2. However, if an individual is diagnosed without the consecutive OGTTs, the following also constitutes diagnosis in TrialNet:
            1. Diabetic OGTT + FPG ≥ 126 mg/dl [7 mmol/L] not on the same day
            2. Diabetic OGTT + HbA1c ≥ 6.5% (these may be on the same day)
            3. FPG ≥ 126 mg/dl [7 mmol/L] + HbA1c ≥ 6.5% (these may be on the same day)

It is preferred that OGTTs for Stage 3 T1D diagnosis are analyzed using TrialNet laboratories. If a participant has an OGTT that indicates diabetes (clinical alert), the site should invite the participant for a repeat OGTT as soon as possible. To make a diagnosis of Stage 3 T1D diabetes, each of two consecutive OGTTs must meet diabetes criteria. Thus, if the second OGTT does not confirm the diagnosis, the participant will continue to be followed in the ATG Low-Dose Prevention Study and will be asked to return for the next follow-up visit. At that time, the OGTT will be repeated and, if indicative of diabetes, will need to be performed a second time to confirm the diagnosis.

In the case of symptoms or unequivocal hyperglycemia with acute metabolic decompensation (diabetic ketoacidosis), repeat testing is not required to confirm the diagnosis of Stage 3 T1D and prompt evaluation and initiation of clinically indicated treatment will occur.

The TrialNet Eligibility and Events Committee will review and adjudicate any cases with only a single objective measure, cases which do not meet the criteria above, and/or cases which have uncertain results of tests and/or the test procedures. Qualifying glucose values should be from laboratory tests, rather than capillary blood glucose meter readings.

The study design is a 2:1 randomized, double-blind placebo-controlled trial. The primary objective of this trial is to assess the efficacy of ATG versus placebo control in terms of the risk of diabetes onset in this target population as defined by the eligibility criteria.

The cumulative incidence of diabetes onset over time since randomization within each treatment group will be estimated using the Kaplan-Meier method (proportion surviving diabetes-free as a function of time). The difference between groups in the cumulative incidence functions, and the associated hazard functions, will be tested at the 0.05 level, two-sided, using the Cox Proportional Hazards (PH) model, adjusted for age. Assumptions of proportionality will be tested, and if that assumption does not hold, then alternative analytic approaches will be used to allow for time-varying coefficients in the model. The relative risk of diabetes onset between groups will be estimated from the Cox regression model. The critical values will be determined by the group-sequential procedure outlined in the section entitled Interim Monitoring Plan below.

8.2 Secondary Outcome and Analyses

A variety of secondary analyses are planned, some of which will include the following :

- Subgroup analyses may be conducted comparing the effects of low-dose ATG versus control on the risk of diabetes within subsets of the study cohort. Subgroups of the population may be classified by age, gender, race/ethnicity, specific antibody status at baseline, and HLA status (e.g. presence or absence of HLA DR3/DQ2 and/or HLA DR4/DQ8). Differences in the treatment effect between subgroups will be tested using a covariate by treatment group interaction term in the Cox regression model.
- The heterogeneity of treatment effect across categorical factors will be assessed through a covariate by treatment group interaction in a Cox hazards regression model. These factors include gender, race/ethnicity, specific antibody status at baseline and HLA status (i.e. presence or absence of HLA DR3/DQ2 and/or HLA DR4/DQ8).
- Similarly, subset analyses will be conducted using the values of quantitative baseline factors (dichotomized at the median) Including weight, BMI-Z, CBC and the immunologic and metabolic factors described in Section 5 that include autoantibody titers, basal c-peptide, OGTT stimulated c-peptide (peak and AUC-mean).
- Additional covariates may be defined during the conduct of the study. The reporting

of the analyses will distinguish between factors specified prior to primary analysis and those identified post-hoc during analysis.

- Longitudinal analyses will assess the effects of low-dose ATG versus control treatment on immunologic and metabolic markers over time up and following progression to stage 3 T1D. Differences between groups in the mean levels of quantitative factors over time will be assessed using a normal errors linear model for repeated measures. Differences between groups in the prevalence of qualitative factors over time will be assessed using generalized estimating equations for categorical measures. Generalized estimating equations may also be employed for the analysis of quantitative factors when the normal errors assumptions are violated.
- The association of demographic, genetic, immunologic, metabolic, and lifestyle factors, among others, both at baseline and over time, with the risk of diabetes onset will be assessed in Cox regression models over time. The effects of changes in longitudinal factors on diabetes risk will be assessed using time-dependent covariates for these factors. Analyses will be conducted separately within the treatment and control groups, and differences between groups in covariate effects (group by covariate interactions) will be assessed. Longitudinal analyses of C-peptide AUC means 3, 6, 9, 12, 18, 24, 30, 36, 42, 48, 54 and 60 months using a mixed effects model with a random intercept and slope by participant, adjusted for the baseline level of C-peptide, gender and baseline age. The mean intercept and slope will be compared between treatment groups.
- Toxicity and tolerability will also be assessed in all participants who have received at least one dose as part of this trial. Toxicity is defined as an adverse event that is classified as either possibly, probably, or definitely related to study treatment. The maximum grade for each type of toxicity will be recorded for each participant, and frequency tables will be reviewed to determine toxicity patterns. In addition, we will review all adverse event data that is graded as 3, 4, or 5 and classified as either “unrelated” or “unlikely to be related” to study treatment in the event of an actual relationship developing.
- The incidence of severe (grade 3+) adverse events or toxicities will be described for each treatment arm but will also be compared between the arms. Fisher’s exact tests will be used to quantitatively compare the incidence of severe as well as specific toxicities of interest between the treatment arms, and we will graphically assess differences in maximum grades observed for toxicities between the arms. Fisher’s exact test on adverse events will be used for descriptive purposes only; p-values provided will be nominal p-values (there is no correction for multiplicity planned, so no inferential conclusion can be drawn from there). These analyses will be useful in identifying potentially toxicity patterns of interest and in planning future trials. We will also assess tolerability of low-dose ATG through assessing the proportion of people who discontinue treatment due to adverse reactions or even those who refuse further treatment for lesser toxicities that inhibit their willingness to continue participation on the trial. These tolerability measures will be assessed within each of the treatment arms and we will explore differences in these measures

between the arms. All participants who have received at least one dose of any of the therapeutic agents in a treatment arm will be evaluable for toxicity and tolerability.

Additional secondary objectives to determine the effect low-dose ATG on metabolic and immunologic measures such as but not limited to:

- HbA1c over time by treatment group using ANCOVA.
- Index 60
- AUC Glucose post OGTT
- CGM variables and metrics
- Descriptive Assessment of Safety and Tolerability of Adverse events to include but not limited to:
    - Number and severity.
    - The rates of severe adverse events will be computed (total number of events divided by total participant years of follow-up)
- CD4:CD8 panel

8.3 Additional Outcomes and Analyses

Additional outcomes of interest include the effects of low-dose ATG vs placebo control treatment with regard to the Mechanistic Studies assessed from blood draws as outlined in the Schedule of Assessments. Additional analyses will compare the results in this trial to other TrialNet prevention studies. Data in this trial will be used in conjunction with other TrialNet data for exploratory analysis.

8.4 Sample Size and Power Calculations

This study has been designed to provide at least 85% power to detect a 60% risk reduction in the hazard rate for progression from Stage 2 (with a 50% 2 year progression risk) to Stage 3 T1D using a two-sided test at the 0.05 level once the targeted number of Stage 3 T1D's have been observed. We note that if accrual is more rapid than expected, minimum follow-up on participants may need to be extended or additional accrual in order to meet the number of events required for the final analysis.

A total of 101 participants will be randomized in a 2:1 allocation to treatment with low-dose ATG vs. placebo (e.g., 68 and 33, respectively). Randomization will be conducted using block randomization with variable block sizes.

The assumptions underlying the estimated sample size for this design are: (1) a detectable hazard ratio of 0.4, (2) a minimum of 2 years of follow-up on all participants who have not progressed to Stage 3 T1D, (3) an accrual period of approximately 4 years, and (4) less than a 10% two-year dropout rate in both groups. Under the above assumptions, the target total number of events is equal to 44 assuming the alternative hypothesis; i.e., the final analysis will be conducted once 44 events (Stage 3 T1D diagnosis) have been observed.

With this augmented eligibility criteria and based on the TrialNet Pathway to Prevention (PTP) data (TN01), we would expect to have on average 20 participants per year aged 6 to 35 years old identified with two or more of the high-risk features described in the eligibility criteria.

The final test of significance will employ group sequential critical values to protect against inflation in the type I error probability due to interim assessments of the emerging data for review by the DSMB (see Section 8.5).

Note the accrual period and the study sample are only projections, and the actual accrual rate and the loss-to-follow-up rate are unknown. As the study progresses, more accurate projections of the study end date will be computed based on the observed rate of enrollment, the observed number of events, and the observed rate of loss-to-follow-up. These data will be provided to the DSMB and the TrialNet governing body, and if need be, this document will be amended to reflect revised planning parameters.

8.5 Interim Monitoring Plan

8.5.1 Monitoring for an increased rate of progression for individuals treated with ATG

The DSMB will be informed of cases that progress to Stage 3 type 1 diabetes as they occur for the duration of the study. Additionally, the DSMB will meet every 6 months to review whether or not the percent of study participants who progress to Stage 3 type 1 diabetes on the ATG arm deviates by more than 10% from either the TrialNet historical data for a population that mirrors the eligibility of this study or more than 10% from the percent of those who progress on the control arm. The data that support this monitoring will be provided to both the DSMB and the FDA.

8.5.2 Formal Interim Analysis

A formal interim analysis of efficacy will be conducted when 50% of events (i.e., information fraction = 0.50) have been observed; i.e., we will conduct an interim analysis to compare the two treatment arms when 22 participants enrolled on this trial have a reported progression to Stage 3 T1D. To preserve the Type I error rate control for each of these comparisons on superiority, the Lan-DeMets error spending rate function with the O'Brien-Fleming boundaries is utilized. If these boundaries are crossed, then the TrialNet DSMB will determine if accrual to that arm should be suspended and/or if treatment of participants should be modified based on these results. These computations as based on Reboussin et al paper[73].

Information fraction	Cumulative events	Alpha (in terms of the efficacy boundary)	Chi-square Efficacy Boundary* (p-value)
0.50	22	0.00153	8.78 (0.00305)
1.00	44	0.0235	3.88 (0.0489)

* ATG superior direction only

In lieu of a formal futility rule at the interim analysis, we will utilize conditional probability methods to assess the probability that low-dose ATG will delay time to T1D based on the data observed

(i.e., 50% information). The DSMB will also consider early termination due to absence of a treatment effect based on computations of conditional power conducted under the initial study design. Conditional probabilities of ≤0.2 will be considered strong evidence for early termination.

While the study is proposed to investigate whether treatment with ATG will lead to a reduction in the rate of progression to Stage 3 disease, the design incorporates the question of whether there is a difference in the rate of progression in either direction, i.e., an increase or decrease. The target sample size is based upon a 2-sided test of significance at the 0.05 level to ensure that the study would have 85% power to detect the minimally clinically defined difference in either direction should it occur.

The interim analysis contains two calculations designed to detect an early emerging difference and a separate analysis of study power, conditioned on the events observed at that time. The boundaries for early emerging differences are two-sided, so as to detect a difference in either direction (i.e., a reduction or increase in the rate of progression) should the observed data so indicate. Conditional power calculations are agnostic insofar as direction of a difference. They are based on the probability of detecting any difference, conditioned on the data at hand and a simulation of the expected study outcome, assuming the alternative hypothesis. The protocol explicitly states that if the probability of detect a difference is below threshold, 0.2, then the DSMB may recommend halting the study for futility.

8.6 Withdrawal Criteria – Individual Participants

An intention-to-treat approach will be used in these analyses. Participants will not be replaced. All data acquired prior to termination for the reasons outlined below will be included in the primary analysis unless a participant withdraws consent for the use of those data. Every effort will be made to conduct a final study visit with the participant, and participants will be followed clinically until, if applicable, all potentially study-related adverse events resolve.

- Withdrawal of consent by the participant
- Withdrawal of the participant by the investigator
- Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease.

9 ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

9.1 Statement of Compliance

This study will be conducted in compliance with the protocol and consistent with current Good Clinical Practices (GCP), adopting the principles of the Declaration of Helsinki, and all applicable regulatory requirements (*ICH E6, 45CFR46, and FDA 21CFR sections 11, 50, 56, 312)*.

The study protocol, along with the required informed consent forms, will be approved by a Central Institutional Review Board (CIRB), and/or each participating institution's Institutional Review Board (IRB) or Ethics Committee/Research Ethics Board (EC/REB) at international sites prior to the initiation of any research procedures (at the site). Any amendments to the protocol or consent materials must also be approved before they are implemented.

In addition to details described in the sections above (informed consent, confidentiality, and risks and benefits), the investigators have reviewed and considered ethical ramifications in the design and development of this protocol. The investigators have made every effort to minimize and monitor risks and discomforts to participants throughout the course of the study.

9.2 Participating Centers

Participating TrialNet clinical sites must have an appropriate assurance, such as a Federal-wide Assurance (FWA) or an Unaffiliated Investigators Agreement (UIA), with the Office for Human Research Protections (OHRP), since they are actively engaged in research and provide informed consent. The protocol and consent forms will be approved by a Central Institutional Review Boards (CIRB) or Institutional Review Boards or Ethics Committees/Research Ethics Boards at each of the participating clinical sites. HIPAA and applicable local regulations will be followed by each participating institution in accordance with each institution's requirements. The participating international sites will obtain approval from their corresponding review boards in accordance with their local procedures and institutional requirements.

The investigator is required to keep accurate records to ensure the conduct of the study is fully documented. The investigator is required to ensure that all case report forms are legibly completed for every participant entered in the trial.

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants participating in this study. When a participant participates in this study at more than one TrialNet site, sharing of this information is required. Sharing of information obtained during this study between TrialNet clinical centers, affiliates, TrialNet Hub and TNCC will be done to assure participant understanding and consent, safety, and adherence to protocol. Medical and research records will be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the sponsor(s) and regulatory agencies to examine (and when required by applicable law, to copy) records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded

identity associated with documents or other participant data may be copied (obscuring any personally identifying information). Authorized representatives, as noted above, are bound to maintain the strict confidentiality of medical and research information that may be linked to identify individuals. The investigational site will normally be notified in advance of auditing visits.

9.3 Informed Consent

The process of assuring that individuals (and parent/guardian if less than 18 years of age) are making an informed decision about participating in this study includes both verbal and written communication. Written materials include a Volunteer Handbook, Volunteer Understanding Assessment, and written consent forms. There is one main consent form for this study that includes screening, intervention and follow-up phases of the study that describes the procedures, risks, and benefits, and eligibility. A second consent form (Follow-up Form) is for use at clinical sites that will be performing the post-treatment visits, but not the treatment visits. The consent forms will be reviewed with participants (and their parent/guardian in the case of participants under 18 years of age) and the participant will be given time to review the written consent form and ask questions. An assent form has also been developed for participants less than 18 years of age (unless local IRB/REB requirements differ in procedure).

As part of the informed consent process, the participant and/or parent or guardian (if the participant is less than 18 years of age) will also be required to complete a short, written Volunteer Understanding Assessment that is designed to ensure that the participant understands the study, as well as what is being asked of him/her. The participant will be given a copy of their consent/assent forms.

The consent process will be conducted by qualified study personnel (the Trial Investigator or Study Coordinator and/or as described in the delegation log). All participants (or their legally acceptable representative) must read, sign and date a consent form prior to participation in the study, and/or undergoing any study-specific procedures.

The informed consent form must be updated or revised whenever there is new, clinically significant information applicable to the safety of the participants, when indicated for a protocol amendment, and/or whenever any new information becomes available that may affect a participant's participation in the study.

Participants will be re-consented if they reach the age of 18 years while enrolled in the study.

9.4 Study Participant Confidentiality

Study participant data, which is for reporting purposes, will be stored at the TrialNet Coordinating Center. Data sent to the Coordinating Center will identify participants by the unique TrialNet Identification Number. The data entry system at the Coordinating Center is a secured, password protected computer system. At the end of the study, all study databases will be archived at the Coordinating Center for long-term storage.

Stored samples including genetic samples could be utilized to learn more about causes of type 1 diabetes, its complications (such as eye, nerve, and kidney damage) and other conditions for which individuals with diabetes are at increased risk, and how to improve treatment. The results of these future analyses, and any mechanistic studies will not be made known to the participant.

9.5 Risks and Benefits

The risks of this study are presented in this protocol, and informed consent form. There is no guaranteed benefit to participants for their participation in the study.

Special consideration regarding risks and benefits for children is described in section 6.1.

10 STUDY ADMINISTRATION

10.1 Organizational Structure

This study is part of Type 1 Diabetes TrialNet, which is funded by the National Institutes of Health. Funding will cover the costs of administration and laboratory tests associated with this study.

10.2 Role of Industry

Sanofi will be providing the active agent, ATG for the study.

10.3 Groups and Committees

10.3.1 ATG Study Chair Committee

The Study Chair and TrialNet Executive Committee will receive periodic reports from the TNCC on the progress of the study. These will include accrual rates and baseline demographic characteristics. Interim data summaries provided to others (except those that could lead to unmasking of study outcome) will first be supplied to the Study Chair for review. Criteria and results of ongoing monitoring of the TrialNet labs in terms of reproducibility will also be provided on a routine basis and reported on during TN28 ATG Study Chair Committee meetings, as scheduled. As appropriate, abstracts and manuscripts dealing with the progress of the trial shall be directed by the TN28 ATG Study Chair Committee.

10.3.2 TrialNet Chairman's Office and TNCC

The TrialNet Chairman's Office, TrialNet Hub and TNCC will work together in providing leadership to the TrialNet study group to include protocol and manual preparation, training for clinical sites, development of statistical design for each study, and analysis of study results. The TNCC will also coordinate interactions among the participating TrialNet Clinical Centers, test laboratories including TrialNet Core Laboratories and other subcontract laboratories, NIDDK, and other sponsoring agencies.

10.3.3 Clinical Sites

Principal Investigators at each participating TrialNet clinical site will oversee all operations at that site. The clinical sites will forward all laboratory and data collection form information to the TNCC for analysis. Direct communication and site visits, as needed, will facilitate evaluation of the trial management.

10.3.4 Clinical Site Monitoring

In order to conduct this study with established research principles, site visits will be conducted during the study to evaluate study conduct and ensure participant safety. All sites will be monitored by the TNCC and appropriate TrialNet committees for patient enrollment, compliance with protocol procedures, completeness and accuracy of data entry, the occurrence and reporting of adverse

events (Aes) and serious adverse events (SAEs), site pharmacy accountability/operations and to confirm the presence of appropriate IRB/REB regulatory approvals/documents.

10.3.5 Medical Monitor and Data Safety and Monitoring Board (DSMB)

All adverse events will be recorded on the adverse event forms, which will be sent to the local IRBs/REBs, per their reporting requirements, and to the Coordinating Center.

An independent physician will be designated to serve as the medical monitor for this study who will maintain regular contact with the study and the Study Chair. The medical monitor will review all adverse event reports, masked to treatment assignment, and will file event reports with regulatory authorities as appropriate.

The DSMB will meet approximately every 3 months following randomization of the first participant and as needed to review indicators of safety. In addition, they will meet every 6 months to review the interim effectiveness and potential toxicity of the study treatments based on interim analyses of indicators of effectiveness and safety prepared by the TNCC separately by treatment group. The DSMB will independently evaluate whether there are grounds to modify or discontinue the study.

10.4 Sample and Data Storage

Samples to be stored for research purposes will be located at the NIDDK Repository and at TrialNet Laboratories. While TrialNet is active, the use of the samples will be restricted to TrialNet researchers unless researchers from outside of TrialNet obtain approval from TrialNet and the NIDDK to utilize the samples. All samples will be coded with unique study numbers, but TrialNet researchers will be able to identify samples if it is necessary to contact participants for reasons of health or for notification to them about future studies. Approval from TrialNet would be required before such linkage could occur. Researchers from outside of TrialNet will not be permitted to identify samples.

Data collected for this study will be sent to the TNCC. De-identified data will be stored at the NIDDK Repository, under the supervision of the NIDDK/NIH, for use by researchers including those outside of TrialNet.

With permission of the participant, when TrialNet is completed, samples will continue to be stored at the NIDDK Repository. Since the stored data will be fully de-identified upon the completion of TrialNet, it will no longer be possible to identify samples. Thus, whereas a sample can be destroyed upon a participant's request during the existence of the TrialNet, it can no longer be destroyed once TrialNet is completed. However, there will still be the potential to link data derived from the samples with data that had been derived from TrialNet studies. Once TrialNet is completed, researchers will only obtain access to samples through grant proposals approved by the NIDDK. The NIDDK will convene an external panel of experts to review requests for access to samples.

10.5 Preservation of the Integrity of the Study

The scientific integrity of the trial dictates that results be reported on a study-wide basis; thus, an individual Center will not report the data collected from its site alone. All presentations and publications using TrialNet trial data must protect the main objectives of the study. Data that could be perceived as threatening the study outcome will not be presented prior to release of the primary study outcomes. Approval as to the timing of presentations of data and the meetings at which they might be presented will be granted by the TrialNet Steering Committee. Study results should be discussed with the news media only upon authorization of the Steering Committee, and never before the results are presented. Any written statements about this study that are shared with national media must be approved by TrialNet before release.

10.6 Participant Reimbursement and Compensation

Participants may be compensated for each visit attended in the study. In compliance with ICH Guidance E6, the amount and method of payments to participants shall be designed to avoid coercion or undue influence on the study participants. Payments to participants will be prorated and not wholly contingent on completion of the trial by the participant.

APPENDIX 1 – Schedule of Assessments

Week of Trial						**1**	**2**											
Month of Trial								**3**	**6**	**12**	**18**	**24**	**30**	**36**	**42**	**48**	**54**	**60[16]**
Day of Trial		**0**	**1**	**2**	**4**	**7**												
Visit number	**-1**	**0**	**1**	**1.1**	**1.2**	**1.3**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Visit Window		**≤ 52 days stage 2 criteria + 1 or more high risk markers**	**≥ 12 hrs to ≤ 30 hrs from start dose 1**			**+/- 1 day**	**+/- 3 days**	**+/- 7 days**	**+/- 14 days**									
Informed Consent	X																	
Randomization		X																
ATG/Placebo Infusion 1 and 2		X	X															
Medical History	X	X																
Adverse Events Assessment		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam[1]	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X
Urine Pregnancy Test	X	X					X	X	X	X	X	X	X	X	X	X	X	X
TB Test (IGRA) (local)	X																	
CD4/CD8 Panel[2] (local)	X	X	X				X	X	X	X								
CBC with Differential[3] (local)	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X
Chemistries and liver function tests	X	X	X				X	X	X	X	X	X		X		X		X
PT/PTT/INR (local)		X	X															
EBV/CMV viral load and serology[4]	X									X								
HIV, Hepatitis B and C serology	X																	
COVID Test[5] (local)		X																
Random Glucose (local)[6]		X																
OGTT[7]	(X)[8]							X	X	X	X	X	X	X	X	X	X	X
HbA1c	(X)[8]							X	X	X	X	X	X	X	X	X	X	X
Autoantibodies	(X)[8]																	
ATG Levels[9]		X	X	X	X	X	X	X										
ATG Drug Induced Antibodies (DIA)[10]		X			X	X	X	X										
Mechanistic Assessments[11]		X	X				X	X	X	X	X	X	X	X	X	X	X	X
Interim Contact[12]		X			X		X	X	X	X	X	X	X	X	X	X	X	X

Week of Trial						**1**	**2**											
Month of Trial								**3**	**6**	**12**	**18**	**24**	**30**	**36**	**42**	**48**	**54**	**60**[16]
Day of Trial		**0**	**1**	**2**	**4**	**7**												
Visit number	**-1**	**0**	**1**	**1.1**	**1.2**	**1.3**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Visit Window		**≤ 52 days stage 2 criteria + 1 or more high risk markers**	**≥ 12 hrs to ≤ 30 hrs from start dose 1**			**+/- 1 day**	**+/- 3 days**	**+/- 7 days**	**+/- 14 days**									
Flu Vaccination[13]																		
CGM Blinded Wear [14]	X							X	X	X	X	X	X	X	X	X	X	X
Fecal Sample[15]		X						X		X								

1. Complete physical examination at screening and annually thereafter. Interim medical history and directed/limited physical examination every visit. Tanner Staging to occur at screening or baseline visit and annually thereafter. Once Tanner Stage assessed as ≥ 3, no further genitalia examination required. Directed exam as indicated by symptoms.
2. CD4/CD8 panel will continue to be monitored until the CD4 count is above 500. CD4/CD8 ratio can be collected at screening visit or baseline (Visit 0).
3. CBC to be obtained prior to the first infusion, immediately following the first infusion, prior to the second infusion and obtained after the completion of the second infusion.
4. Viral PCR/Serology: All participants will have serology and PCR for EBV and CMV at screening. CMV and/or EBV seronegative participants must be PCR negative, CMV seropositive participants must be CMV PCR negative, and EBV seropositive participants must have EBV PCR <2000 IU/mL within 30 days of randomization. All participants may not have had signs or symptoms of an EBV or CMV compatible illness lasting longer than 7 days within 30 days of randomization. Additional EBV and/or CMV PCR testing may be obtained locally if necessary clinically. Subsequent EBV and/or CMV PCR/Serology will only be performed for symptomatic participants as clinically indicated. At Month 12, EBV/CMV serology will be processed real-time and PCR for EBV/CMV will be stored unless clinically indicated.
5. Covid-19 test to be done within 7 days of the first dose (infusion 1) to ensure no active infection is present before treatment administration. Additional Covid-19 testing will be performed at specified visits based on exposure, symptoms and investigator's recommendation.
6. Random glucose to be obtained prior to randomization to monitor progression to Stage 3 T1D. If value is less than 200, randomization can proceed. If value is above 200, a safety check must be done by the study physician for Stage 3 T1D.
7. If OGTT consistent with DM, repeat as soon as possible but no less than one day apart. Aim to repeat within 1 month. Mechanistic samples, including CBC/differential will be collected at the confirmatory OGTT visit.
8. If not done as part of the TrialNet Pathway to Prevention study or other TrialNet Prevention study, OGTT, HbA1c and Islet Autoantibodies may be done at the screening visit.
9. ATG Levels (PK): Samples will be drawn on 12 participants in each of the following age groups: <18 years and ≥18 years at randomization. Day 0 samples to be drawn before infusion 1 and 6 hours after the completion of infusion 1. Day 1 samples to be drawn before infusion 2. Additional samples will be collected on Days 2, 4, and 7, Week 2, and Month 3.
10. ATG Drug Induced Antibodies (DIA): Samples will be drawn on 12 participants in each of the following age groups: <18 years and ≥18 years at randomization. Pre-infusion sample will be drawn on Day 0. Samples will also be drawn on Days 4 and 7, Week 2, and Month 3.
11. May include samples for RNA, plasma, serum, DNA, measures of B and T cell number and function to understand the effect of therapy on the immune system and infectious disease. The following HLA typing will also be performed: Class I (HLA-A, B, C) and Class II (DRB, DBA, DQB). The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the participant's age and body weight (for participants <18 years, 5 mL/kg per visit, 9.5 mL/kg in an 8-week period).
12. Contact with participant every other day will occur once the study treatment is complete until the 2-week visits. Following the 2-week visit weekly contact with participant will occur until the 3-month visit. Contact thereafter will occur every 3 months, in-between in person study visits to assess for symptoms of diabetes and changes in health. Participants who report signs/symptoms consistent with Stage 3 T1D will be asked to complete a PRN OGTT study visit. At any time point in between study visits, participants experiencing symptoms of diabetes are to contact their clinical trial contact(s). Covid-19 assessments may be completed PRN through the end of the study based on exposure and symptoms.
13. Participants are required to receive killed influenza vaccination at least 2 weeks prior to randomization when vaccine for the current or upcoming flu season is available.
14. Frequency of CGM blinded, 7-10 day wear will occur at the screening visit, 3 month visit and then semi-annually per schedule of visits.
15. Fecal sample: Willing participants will collect fecal samples within +/-3 days of the indicated visit at home and either bring to the research visit or ship samples back to the site. Participants who agree to collect samples should be encouraged to provide samples at all 3 timepoints.
16. The bi-annual schedule will continue until the primary endpoint of the study occurs. After the primary endpoint is met, all participants will be followed for an additional year. Participants who develop Stage 3 T1D at any point during the year post-primary endpoint, will be followed for 1 year following their diagnosis.

APPENDIX 2 - Schedule of Assessments for Participants Who Progress to Stage 3 T1D

	Initial[1]				
Month		**6**	**12**	**18**	**24+[6]**
Visit Window	**+/- 7 days**	**+/- 14 days**	**+/- 14 days**	**+/- 14 days**	**+/- 14 days**
Directed Physical Exam	X	X	X	X	X
Intensive Diabetes Management Review	X	X	X	X	X
MMTT[2]	X	X	X	X	X
HbA1c[2]	X	X	X	X	X
Concomitant Medication	X	X	X	X	X
Adverse Event Assessment	X	X	X	X	X
Urine Pregnancy Test	X	X	X	X	X
Mechanistic Assessments[3]	X	X	X	X	X
Unblinded CGM Wear[4]	X	X	X	X	X
Fecal Sample[5]					

1. Initial Visit: To occur within 12 weeks from date of diagnosis of Stage 3 T1D
2. MMTT to be performed only when residual C-Peptide is evident at last available MMTT result. If c-peptide is no longer detectable at last available MMTT result, the participant will no longer complete MMTTs. From this point, the participant will only complete the other visit assessments, including HbA1c, which can be done in-person or remotely. Some individuals with c-peptide may decline undergoing MMTT visits but still wish to participate in the study. In this case visit assessments may be obtained in person or remotely.
3. Samples may be obtained for mechanistic studies developed to address the aims of this protocol, including RNA, plasma, serum, DNA, and/or measures of immune cell number and function. At no time will blood volume exceed what is allowable per the participant's age and body weight (5 mL/kg per day and 9.5 mL/kg over an 8-week period for children under age 18.
4. Unblinded CGM wear for 7-10 days will occur after each visit in the Stage 3 T1D schedule.
5. If participant is diagnosed with Stage 3 T1D <1 year after baseline (V0), fecal sample can be collected at a stage 3 visit if the visit occurs 3 months ±7 days and/or 12 months ±14 days after baseline (window based on the Stage 2 schedule for V3 and V5).
6. The bi-annual schedule will continue as long as follow-up continues within the scope of this trial.

APPENDIX 3: Criteria for the Diagnosis of Diabetes[7]

A1C ≥6.5% (≥48 mmol/mol). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*
OR
FPG ≥126 mg/dL (≥7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.*
OR
2-h PG ≥200 mg/dL (≥11.1 mmol/L) during OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*
OR
In an individual with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (≥11.1 mmol/L). Random is any time of the day without regard to time since previous meal.

DCCT, Diabetes Control and Complications Trial; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; NGSP, National Glycohemoglobin Standardization Program; WHO, World Health Organization; 2-h PG, 2-h plasma glucose. *In the absence of unequivocal hyperglycemia, diagnosis requires two abnormal test results obtained at the same time (e.g., A1C and FPG) or at two different time points.

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